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COLORIMETRIC ASSAY OF TETRACYCLINE ANTIBIOTICS

BY

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Colorimetric Assay of Tetracycline Antibiotics" submitted by Sherry Irene Krause in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The color developed when compounds of the tetracycline series are complexed with thorium has been utilized as the basis for a quantitative analytical procedure for these drugs. In addition to detailed studies regarding the effects of time and pH on the stability of the colored complex, a structure for the complex has been postulated.

For all the tetracyclines investigated, linear and reproducible calibration curves have been readily obtained and the technique was subsequently applied to 29 different pharmaceutical dosage forms. For most of the products assayed, good agreement has been observed between the results obtained by the present method and those supplied by the pharmaceutical manufacturers according to their procedures.

Preliminary investigations have been made regarding the applicability of nuclear magnetic resonance spectroscopy to the quantitative analysis of the tetracycline compounds. The use of maleic acid as a suitable internal standard in the NMR method of analysis of these antibiotics has been suggested.

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Squibb & Sons, Limited (Resteclin ® Aqueous Drops, Resteclin ® Capsules, and Resteclin ® Syrup); and the Upjohn Company of Canada (Albamycin ® T Capsules).

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TO RICHARD

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INTRODUCTION

I. Activity and Structural Similarities of Tetracycline Antibiotics

In 1948, a search stimulated by the overwhelming clinical significance of the antibiotics, penicillin and streptomycin, resulted in the discovery of a new chemotherapeutic agent known as chlortetracycline. Within twenty years, a complete series of tetracyclines, similar in structure and activity to the original chlortetracycline, has evolved. The tetracyclines have been found highly effective against rickettsiae and a large number of gram positive and gram negative microorganisms, and have thus earned the designation of "broad-spectrum" antibiotics.

Numerous theories have been formulated to explain the broad antimicrobial action of tetracyclines. Three of the most acceptable suggestions summarized by Goodman and Gilman (1) are that the antibiotics exert their effect by means of:

(1) active chelation of cations,

(2) inhibition of essential enzyme systems,

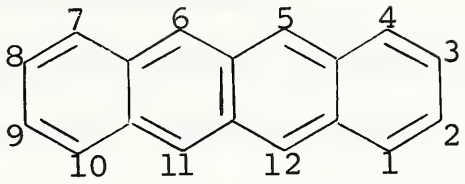
or (3) suppression of protein synthesis.

Despite the plausibility of each of these theories, the exact mechanism of action of these drugs is yet unknown.

Advantages of the use of tetracyclines as chemotherapeutic agents are their low toxicity, their oral efficacy, and their broad-spectrum activity.

Structural Formulas

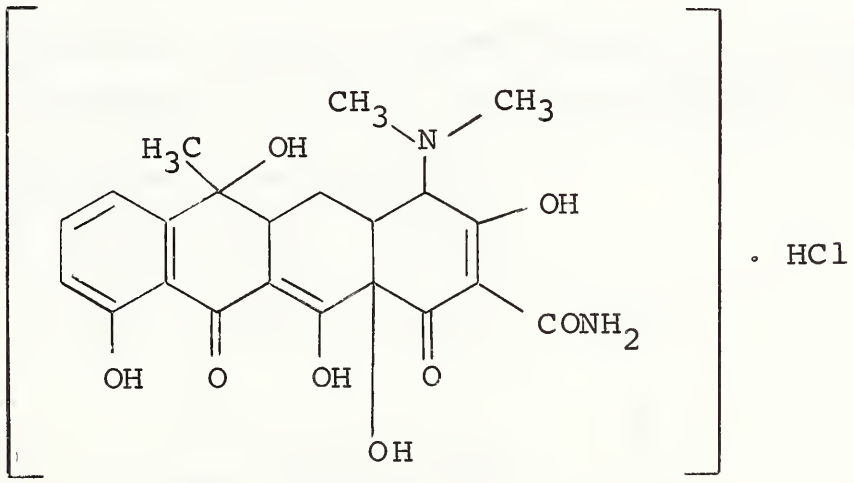
Basic naphthacene ring structure:



(a) Tetracycline Hydrochloride, B.P. (the hydrochloride of 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide).

Molecular weight (base) - 444.43.

Molecular weight (hydrochloride) - 480.9.

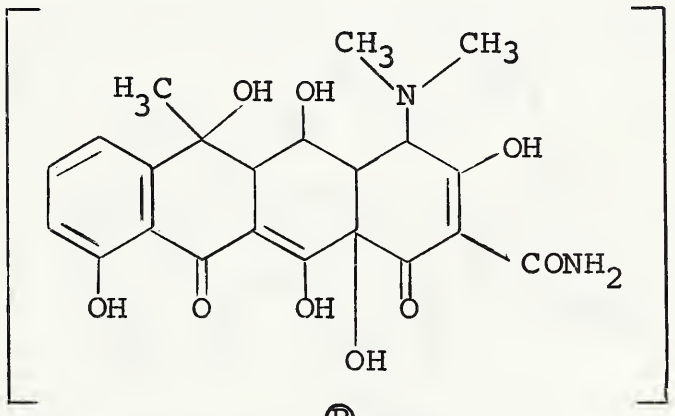


Trade Names: Achromycin®(Lederle), Cefracycline® (Frosst), Decycline®(Desbergers), Double-T®, Tetrasol®, (Horner), Hostacycline-500® (Hoechst) Muracine®(Nadeau), Neotetrine®(Neo, Will), Novotetra®(Novopharm), Tetra-biotic®(Can. Pharm. - Nordic), Tetracyn®(Pfizer).

(b) Oxytetracycline Hydrochloride, B.P. (the hydrochloride of 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide).

Molecular weight (base) - 460.43.

Molecular weight (hydrochloride) - 496.9.



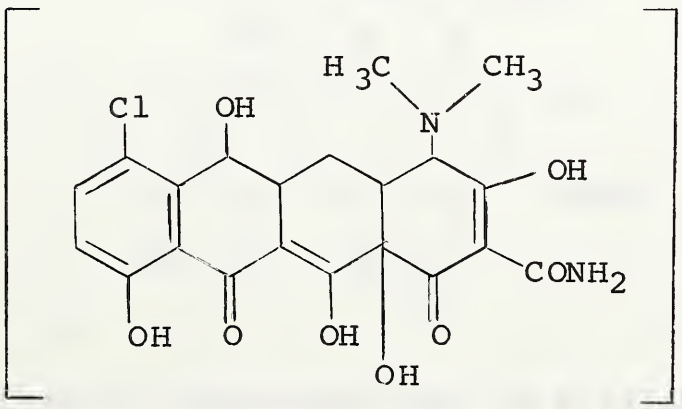
· HCl

Trade Name: Terramycin® (Pfizer).

(c) Demethylchlortetracycline Hydrochloride, B.P. (the hydrochloride of 7-chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxonaphthacene-2-carboxamide).

Molecular weight (base) - 464.8.

Molecular weight (hydrochloride) - 501.3.



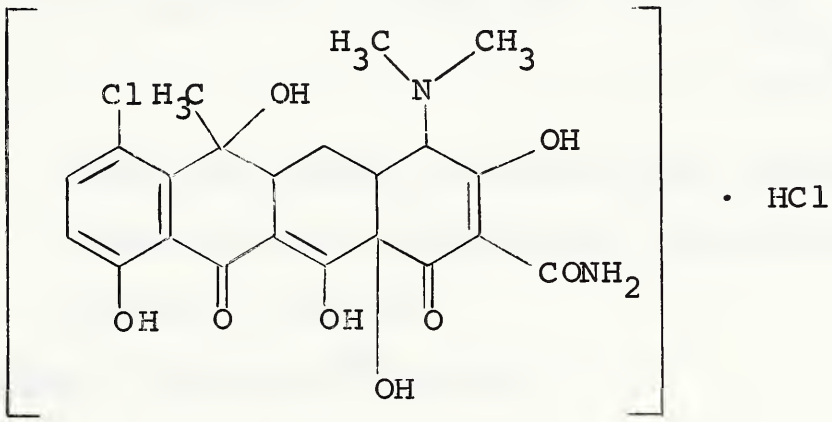
· HCl

Trade Name: Declomycin® (Lederle).

(d) Chlortetracycline Hydrochloride, B.P. (the hydrochloride of 7-chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide.

Molecular weight (base) - 478.88.

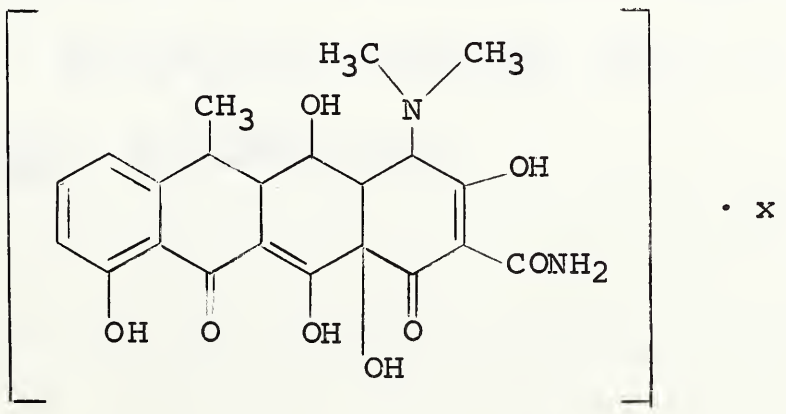
Molecular weight (hydrochloride) - 515.4.



Trade Name: Aureomycin® (Lederle).

(e) Doxycycline (4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide).

Molecular weight of doxycycline base - 444.44.



This recently developed drug is available in three forms:

(1) the monohydrate in which the "x" is H_2O. Molecular weight of doxycycline monohydrate - 462.43.

(2) the hydrochloride in which the "x" is HCl. Molecular weight of doxycycline hydrochloride is 480.9.

(3) the hyclate in which the "x" is HCl
1/2 H_2O
1/2 C_2H_5OH

Molecular weight of doxycycline hydrochloride hemiethanolate hemihydrate (doxycycline hyclate) is 512.94.

Trade Name: Vibramycin®(Pfizer).

(f) Tetracycline Phosphate Complex (tetracycline sodium hexametaphosphate complex).

The precise structural formula of this complex is not known since the complexes vary in size (2). An approximate empirical formula for tetracycline phosphate complex (3) may be denoted as follows:

$$\left[(C_{22}H_{24}N_2O_8)\ Na(PO_3)_6\right]_x \cdot H_2O$$

Trade Name: Tetrex®(Bristol).

II. Analytical Techniques

Since the introduction of tetracyclines to the medical world in 1948, their uses in the field of antibiotic therapy have increased tremendously. Today, this class of broad-spectrum antibiotics dominates in applicability and frequency of use even over such popular antibiotics as penicillin and streptomycin. At present, in Canada, tetracycline products constitute 23.3% of all generic drugs prescribed by medical practitioners (4). Such widespread utilization of these products necessitates the development of new, more rapid and accurate methods of quantitative estimation than have previously been employed.

Maintained by the B.P. 1968 (5) as the official method for the assay of antibiotics, the microbiological assay serves as the standard method employed by pharmaceutical manufacturers to ascertain the potency of their tetracycline products. In this method, the potency of an antibiotic sample is determined by means of a comparison between the dose inhibitory to the growth of a selected, susceptible microorganism, and the dose of the standard preparation of that antibiotic which yields the same degree of inhibition. However, despite the popularity of the microbiological assay for tetracyclines, there are several major factors which make its usefulness and reliability somewhat suspect. First of these is the failure of the procedure to produce consistently precise results. Further, results obtained may in some instances be fallacious as has been shown by Garrett

(6) in a study illustrating that the degradation products of tetracycline, although not possessing the same pharmacological properties as the drug itself, can give anomalous estimates of potency. This is attributed to the fact that the official cylinder plate assay is not only dependent upon the minimum inhibitory concentration in an agar medium, but also upon the diffusibility of the antimicrobial agent through the agar. Thus, if the diffusibility of the degradation product is higher than that of the drug itself, the possibility arises that a more degraded, less potent, sample of tetracycline might yield higher results according to a microbiological assay. Not the least among the problems of a bio-assay for tetracyclines is the time required for such a procedure, since incubation time alone for inoculated agar plates is a period of sixteen hours.

Numerous attempts have been made to develop more rapid methods with increased precision. Among these have been several paper chromatographic procedures, including those of Selzer and Wright (7), who employed buffered paper and mixed solvent systems, and Kelly and Buyske (8) who made use of sequestering agents. Addison and Clark (9) found buffering of the chromatographic paper unnecessary and achieved quite favorable results when utilizing Whatman's modified cellulose phosphate cation-exchange paper. Somewhat more recently, Ascione, Zagar and Chrekian (10) have had a considerable degree of success in the separation and determination of tetracyclines by column chromatography on

EDTA-impregnated diatomaceous earth.

In addition to chromatographic procedures, several other techniques, including non-aqueous titrimetry and fluorometry, have proven to be useful. Sideri and Osol (11) have described the titration of several tetracyclines in glacial acetic acid, while Yokoyama and Chatten (12) were also able to obtain quite satisfactory results with a more complex non-aqueous system composed of nitromethane, formic acid, and benzene. However, a few problems were encountered in the application of the latter method to certain pharmaceutical dosage forms such as ointments and suppositories. In his work with tetracycline antibiotics, Kohn (13) employed fluorometry to take advantage of the ability of tetracyclines to form fluorescent extractable complexes with calcium and barbiturates. This last procedure yielded precise results for the determination of tetracyclines, but was not applicable to oxytetracycline.

A large proportion of the chemical methods currently used for the assay of tetracyclines revolves about their spectral behavior in the ultraviolet region of the spectrum. Chiccarelli and Woolford (14) have described a procedure based upon the changes in the ultraviolet absorption of tetracycline in acidic and basic aqueous media. Continuing with work in this area, Chiccarelli et.al. (15) noted the feasibility of a method for tetracycline and chlortetracycline which was dependent upon the formation of anhydro compounds and the fact that chlortetracycline heated at

pH 7.5 is cleaved to isochlortetracycline while tetracycline under these conditions is relatively stable.

Colorimetry is yet another technique which lends itself to the quantitative analysis of tetracyclines. Colored derivatives or complexes can frequently be formed by the treatment of tetracyclines with appropriate reagents. Some procedures which have been developed involve the measurement of the optical density of a tetracycline solution after combination with iron (III) ions (16,17), with ammonium molybdate (18,19), with diphenylborinic acid (20), with sodium tungstate (21), and most recently with orthomethylbenzenestibonic acid (22).

In 1955, Japanese researchers, Sakaguchi and Taguchi (23) noted that tetracycline forms yellow-colored metal chelate compounds with quadrivalent thorium. Based upon this fact, a colorimetric procedure was developed at that time for the determination of chlortetracycline, using thorium nitrate, $Th(NO_3)_4$, as a reagent. Chlortetracycline, when present in aqueous solution to the extent of 5-40 µg/ml could be determined after the solution had been neutralized with sodium hydroxide, buffered to pH 4.0 with sodium acetate buffer, and subsequently treated with the appropriate amount of thorium nitrate reagent. The absorbance of the solution was read at 405 millimicrons after a twenty minute period and compared with that of a standard. Sakaguchi et.al. (24) further expanded the utility of their method in 1957 by applying it to the quantitative estima-

tion of tetracycline and oxytetracycline, the thorium chelates of which were shown to exhibit maximum absorbances at 395 millimicrons and 385 millimicrons respectively, at pH 4.4.

STATEMENT OF THE PROBLEM

As a result of widespread importance of the tetracycline antibiotics, it has become essential that newer, more sensitive chemical procedures be devised for their quantitative analysis. In the present work, efforts have been made to develop a rapid and specific colorimetric procedure, somewhat simpler and more accurate than those presented to date. Thorium nitrate, previously employed by a group of Japanese workers is also the color-inducing reagent in this method. While the Japanese investigators applied their technique only to some of the existing tetracyclines and to a few of their pharmaceutical dosage forms, it has been the purpose of this study to develop a new method and subsequently to apply it to seven compounds of the tetracycline series and a large number of their pharmaceutical dosage forms. In addition, attempts have been made to investigate the effects of both pH, and quantity of thorium nitrate reagent upon color development, and also to observe the effect of time upon the stability of the colored complex formed. Where possible, the results of the chemical assays performed have been compared with those obtained by the manufacturers' analytical procedures. The structure and nature of the complex formed between thorium and the individual tetracycline antibiotics has been postulated.

A preliminary study has been conducted regarding the utility of nuclear magnetic resonance spectroscopy as a tool to be employed in the quantitative analysis of the

tetracycline antibiotics. A recommendation for future work in this field has resulted.

EXPERIMENTAL

Apparatus

Standard laboratory glassware; Beckman Model B Spectrophotometer; Beckman Zeromatic II pH Meter fitted with glass-calomel electrode system; water bath; hot plate; Buchner funnel (5 cm diameter); Mettler Gram-atic balance; titration light; magnetic stirring apparatus; Tygon tubing.

Reagents and Solutions

All chemicals employed in this investigation were either A.C.S. or reagent grade in quality.

Ethanol; chloroform; glacial acetic acid; 0.05 N perchloric acid in dioxane standardized against potassium acid phthalate; 0.5% crystal violet in glacial acetic acid; 0.5% thorium nitrate (aqueous); 0.5% thorium nitrate (ethanolic).

Reference Standards

The following is a list of the pure drugs employed as reference standards in this investigation:

Drug	Manufacturer
Tetracycline Hydrochloride	The Upjohn Company of Canada
Oxytetracycline Hydrochloride	Pfizer Company Limited
Chlortetracycline Hydrochloride	Cyanamid of Canada Limited
Demethylchlortetracycline Hydrochloride	Cyanamid of Canada Limited
Tetracycline Phosphate Complex	Bristol Laboratories of Canada Ltd.
Doxycycline Hydrochloride	Pfizer Company Limited
Doxycycline Monohydrate	Pfizer Company Limited

Procedures

A. Determination of Reference Standard Purity

The method devised by Sideri and Osol (11) was employed to determine the purity of reference standards. A quantity (50-350 mg) of the crystalline salt was accurately weighed and dissolved in 80 ml of glacial acetic acid. To this solution, 10 ml of 6% mercuric acetate in glacial acetic acid and two drops of 0.5% crystal violet in glacial acetic acid were added. With the aid of a magnetic stirring apparatus and a titration lamp, the solution was first stirred for a five minute period and then titrated to a blue-green endpoint with 0.05 N perchloric acid in dioxane. The progress of the titration was also followed potentiometrically with a Beckman Zeromatic II pH Meter equipped with a glass-calomel electrode combination.

B. Determination of Wavelength of Maximum Absorbance

Using a 1 ml graduated pipette, 0.6 ml of a solution of the tetracycline reference standard, the concentration of which was equal to 250 µg/ml, was introduced into a 10 ml volumetric flask. To this, 0.5 ml of 0.5% thorium nitrate was added, immediately followed by sufficient distilled water to make the volume equal to 10 ml. The flask was shaken for two minutes after which a portion of the solution was transferred to a Beckman cuvette to be scanned against a blank contained in a matching Beckman cuvette. The blank was prepared identically to the sample

solution, excluding the addition of thorium nitrate reagent. The wavelength of maximum absorbance was obtained to the nearest 5 mμ and used in all subsequent readings which involved that particular tetracycline.

C. Examination of Effect of pH on Color Development

All solutions were prepared identically to those in Procedure B with the exception that the final thorium nitrate-tetracycline mixture was diluted to a 10 ml volume with buffers ranging in pH from 2.0 to 6.0. Absorbances were then measured as in Procedure D.

D. Determination of Minimum Time for Optimum Color Development and Stability

To determine the least time necessary for maximum color development, the blank and sample solutions were prepared in the same way as previously recorded for Procedure B. However, the absorbances were determined at five minute intervals from the point of addition of the thorium nitrate reagent. All readings were made at the wavelength previously determined to give optimum absorbance for the particular tetracycline compound.

E. Preparation of Calibration Curves

All calibration curves were prepared in triplicate and all absorbance readings were corrected to account for the degree of purity of the reference standard being used.

(1) Calibration Curves for Tetracycline, Oxytetra-

cycline, and Demethylchlortetracycline:

A quantity of the hydrochloride salt of the tetracycline under investigation was accurately weighed so that it was exactly equivalent to 25.0 mg of the free tetracycline base. This amount of crystalline material was transferred to a 100 ml volumetric flask to which was also added approximately 50 ml of distilled water. After a ten minute period of stirring, the solution was brought to volume with distilled water and the flask shaken. Into five separate 10 ml volumetric flasks, aliquots of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1.0 ml of the 0.25 mg/ml stock solution were pipetted. To this was added 0.5 ml of 0.5% thorium nitrate, after which the contents of each flask were brought to volume with distilled water, and then shaken manually for two minutes. When a period of twenty minutes from the point of addition of the thorium nitrate had elapsed, each solution was transferred to a Beckman cuvette and the absorbance was measured against a blank having the identical composition but with the exclusion of thorium nitrate and contained in a matching cuvette. Readings were made at 395 mμ for tetracycline and oxytetracycline, and at 405 mμ for demethylchlortetracycline. A second

calibration curve was prepared for oxytetracycline using chloroform as the solvent rather than distilled water.

(2) Calibration Curves for Chlortetracycline Hydrochloride, Tetracycline Phosphate Complex, Doxycycline Monohydrate, and Doxycycline Hydrochloride:

The procedure employed for the preparation of suitable calibration curves for these antibiotics was identical to that used in Part (1) with the exception that aliquots were taken from stock solutions whose concentrations were equal to 0.25 mg of the tetracycline salt or complex per milliliter. Other slight variations were required, namely a forty minute stirring period for doxycycline monohydrate and a thirty-five minute lapse between the addition of thorium nitrate and the measurement of absorbance for the doxycycline compounds. All readings were made at 395 mμ except for those of the tetracycline phosphate complex which were read at 405 mμ. For the latter compound, a factor was also derived by comparison of its calibration curve with that of tetracycline to enable calculation of the equivalent amount of tetracycline base contained in a particular quantity of the phosphate complex.

(3) Calibration Curve for Doxycycline Hyclate:

A supply of doxycycline hyclate reference standard was not available. A calibration curve for this compound was derived by extrapolation of data pertaining to doxycycline hydrochloride.

F. Quantitative Assay of Pharmaceutical Dosage Forms

General Procedure #1 - A suitable quantity of the powdered material was carefully weighed and added to 90 ml of distilled water. A twenty minute period of stirring followed. Upon completion of the stirring, the mixture was filtered under suction through Whatman #1 filter paper, after which the clear filtrate was quantitatively transferred to a volumetric flask. It was then carefully brought to volume with distilled water so that the resulting solution had a concentration accurately known to be 0.25 mg of the tetracycline base or salt per ml of solution. Following the preparation of this "stock" solution, 0.6 ml aliquots of it were placed in each of two 10 ml volumetric flasks. To one of the 10 ml flasks, 0.5 ml of 0.5% thorium nitrate was added and the contents of both flasks were brought to volume with distilled water and then shaken for two minutes. After twenty minutes a portion of the contents of the flask to which the thorium nitrate had been added was transferred to a Beckman cuvette and the absorbance was read against a blank (the solution to which thorium nitrate was not added) contained in a matching cuvette. Absorbances

were read at the wavelength of maximum absorbance for the particular tetracycline under investigation.

General Procedure #2 - A suitable quantity of the liquid preparation was accurately pipetted into a volumetric flask and diluted to volume with distilled water such that a stock solution was prepared having a theoretical concentration of 0.25 mg of the tetracycline base or salt per ml of solution. The remainder of the method was identical to Procedure #1, starting with the words, "0.6 ml aliquots of the solution were placed in each of two 10 ml volumetric flasks...." .

(1) Dosage Forms Assayed

a) Capsules: Albamycin T® Capsules
Resteclin® Capsules
Tetrex® Capsules
Tetrex-bid® Capsules
Tetrex-APC® Capsules
Azotrex® Capsules
Vibramycin® Capsules
Achromycin® Capsules
Aureomycin® Capsules
Declostatin® Capsules

For each product ten capsules were emptied and the contents accurately weighed. In order to remove the contents from the last three types of capsules listed, it was necessary first to soften

the elastic capsule and then split it open longitudinally by slicing through the seam of the capsule along one side. Having removed the powder from the capsules the General Procedure #1 was applicable in every instance.

b) Tablets: Achrocidin® Tablets
Aureomycin® Soluble Tablets
Declomycin® 300 Tablets

For each product, ten tablets were accurately weighed and ground to a fine powder using a mortar and pestle. General Procedure #1 was then directly applicable to all three types of tablets investigated.

c) Syrups: Achrocidin® Compound Syrup
Resteclin® Syrup
Azotrex® Syrup
Tetrex® Syrup
Tetrex-AP® Syrup
Declomycin® Syrup
Signemycin® Syrup

For all products listed above, Procedure #2 was employed. Minor variations were required in the assays of Declomycin® Syrup and Signemycin® Syrup wherein 10 ml and 7.5 ml samples of syrup respectively were used rather than the 5 ml sample as stipulated in the general procedure.

d) Aureomycin® Otic Solution - General Procedure

#1 was employed. However, filtration was not required.

e) Terra-Cortril® Eye-Ear Suspension - A vial of the eye/ear suspension was shaken manually for five minutes after which 1 ml was carefully transferred to a 100 ml volumetric flask. Chloroform was added to make the volume exactly equal to 100 ml. After five minutes of mechanical stirring two 5 ml portions of the resulting solution were pipetted into each of two 10 ml volumetric flasks. To one of the flasks 0.5 ml of 0.5% thorium nitrate in ethanol was added while it was excluded from the other flask. Both were brought to volume with chloroform and the absorbances were read after twenty minutes in the usual manner.

f) Resteclin® Aqueous Drops - General Procedure #2 was used in this instance.

g) Terra-Cortril® Ointment and Aureomycin® Ophthalmic Ointment - General Procedure #1 was applied with the exception that the initial 0.25 mg/ml stock solution was prepared by heating and stirring a suitable accurately known weight of ointment with 90 ml of distilled water prior to filtration and dilution with distilled water to an accurate volume.

h) Vibramycin® Oral Suspension - This product was assayed using a modification of Procedure #2.

The entire contents of the bottle were suspended in 200 ml of distilled water and this mixture was stirred mechanically for 45 minutes. After this period, the magnet was removed and the mixture was brought to a 500 ml volume with distilled water. The suspension was allowed to settle and two 0.3 ml portions of the clear pink supernatant liquid were transferred to each of two 10 ml volumetric flasks. To one of these, 0.5 ml of 0.5% thorium nitrate was added and both solutions were brought to volume with distilled water. The solution to which thorium nitrate had not been added served as a blank and after a thirty-five minute period, absorbances were read in the usual manner.

i) Aureomycin® Surgical Powder - Procedure #1 was employed.

j) Terra-Cortril®-Antibiotic Spray - A short length of Tygon tubing was attached to the spray nozzle of the can and the entire contents of the can were expelled into a 150 ml beaker. With the application of a small amount of heat, the propellant was boiled away. The remaining liquid was transferred to a 1000 ml volumetric flask and was brought to volume with chloroform. Two 0.5 ml portions of this solution were transferred accurately to each of two 10 ml volumetric flasks,

one of which contained 0.5 ml of 0.5% thorium nitrate in ethanol. Both solutions were brought to volume with chloroform and absorbances were read in the usual manner after twenty minutes.

RESULTS AND DISCUSSION

Reference Standard Purity

The nonaqueous titrimetric method of Sideri and Osol (11) was found to be very suitable for the assay of the tetracycline reference standards with the exception of the tetracycline phosphate complex. By utilizing glacial acetic acid as an amphiprotic solvent, the weak basicity of the tetracyclines could be enhanced so that a very distinct visual endpoint was easily observed when using perchloric acid in dioxane as the titrant and 0.5% crystal violet in glacial acetic acid as the indicator. In all of the titrations performed, potentiometric readings were made concurrently to ensure exact determination of the endpoint and hence to permit accurate calculation of the reference standard purity.

A summary of the data contained in Table V (Appendix) is presented in Table I. In this table, a complete list of the reference standards employed in the investigation has been given, together with the percentage purity found and the names of the manufacturers supplying the reference materials. In all instances the purity of the standard was found to be not less than 94.8%. However, it was not possible to determine the purity of the tetracycline phosphate complex since the molecular weight of this complex may vary from one batch to another.

Nature of Complexation

A discussion of the mechanism of complexation between thorium and the various tetracyclines must necessarily be

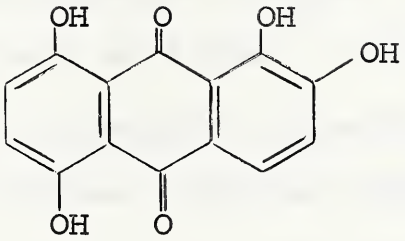
Table I. Reference Standards

Number	Name of Drug	Mol. Wt.	Purity	Supplier
1	Tetracycline HCl	480.9	97.32 %	Upjohn
2	Oxytetracycline HCl	496.9	99.58 %	Pfizer
3	Chlortetracycline HCl	515.4	96.12 %	Cyanamid
4	Demethylchlortetracycline HCl	501.3	97.76 %	Cyanamid
5	Tetracycline phosphate complex	variable	not determined	Bristol
6	Doxycycline HCl	480.9	94.8 %	Pfizer
7	Doxycycline monohydrate	462.4	100.29 %	Pfizer

preceded by a brief mention of the electronic configuration of thorium. As stated in the International Series of Monographs on Analytical Chemistry (25), the electronic structure of thorium has not yet been fully established to the satisfaction of all physical chemists concerned. Dissent has arisen in the elucidation of the ground state electronic configuration in the levels outside the radon structure. The two most generally accepted theories are that these outer levels are either occupied as $6d^2$, $7s^2$ or as 5f, 6d, $7s^2$. In which of the actinide elements the first development of a 5f level occurs is not known. Thus it is very difficult to verify Seaborg's (26) postulate that thorium marks the beginning of the second series of transition elements.

Thorium has been shown to be capable of valencies of two, three or four. In the present study, Th^{+4} is made available for complexation with the tetracyclines as a result of ionic release in aqueous solution from its salt complex, $Th(NO_3)_4$.

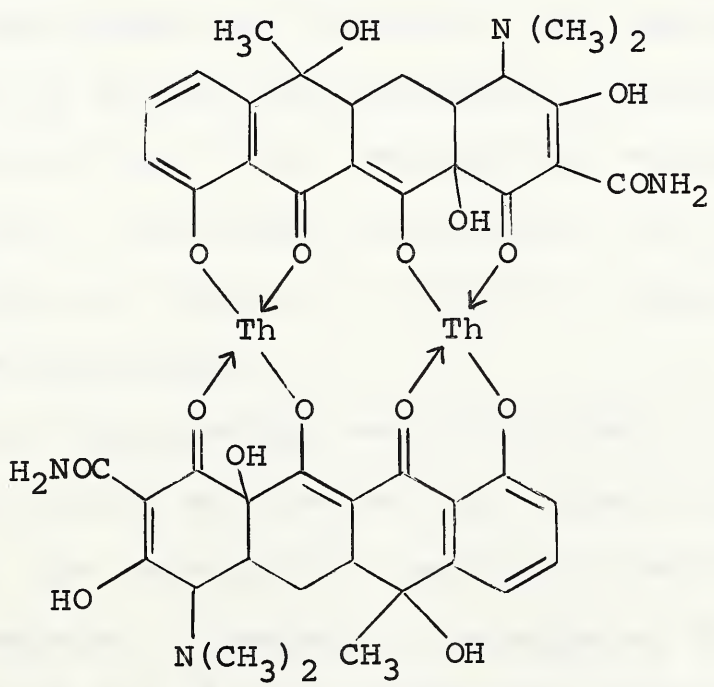
Numerous investigations for analytical purposes have been undertaken to find organic ligands which will form stable complexes with thorium. One of the most useful complexing agents found is quinalizarin, the structure of which is shown on the following page. Quinalizarin has been shown to react with thorium stoichiometrically in a 1:1 ratio in weakly acidic solutions (27). Complexation of thorium with such a compound is generally attributed to the



Quinalizarin

(1,2,5,8-tetrahydroxy-anthraquinone)

availability of oxygen for bond formation. The presence of hydroxyl groups in the ligand leads to the ready formation of a species containing thorium as a complex cation. The similarity of the quinalizarin structure to those of the tetracyclines investigated is apparent. Thus it could be postulated that the type of complex structure formed is analogous in both instances. A general structure for a tetracycline-thorium complex could be as follows:



In this way, a 2:2 coordination compound between tetracycline and thorium would be formed. The molar ratio method was employed to determine the actual combining ratio in the complexation process. From an examination of Figure 1, it can be seen that when the mole fraction of thorium nitrate in the reaction mixture was equal to 0.5, color development and hence complexation, were essentially complete. Thus a combining ratio of 1:1 (or 2:2) between tetracycline and thorium was confirmed.

Position of Absorption Maxima

In the series of tetracyclines investigated, absorption maxima of the uncomplexed drugs in aqueous solution, were found to range from 345 mμ to 365 mμ as shown in Table II. Complexation with thorium invariably resulted in a bathochromic shift of 40 to 50 mμ. This can be attributed to the formation of single covalent and dative bonds between oxygen and thorium in the complexed species, which leads to further delocalization of electrons from the conjugated portions of the tetracycline molecules. Increased stability of the system arising from such delocalization of electrons results in a shift in absorption maximum to a longer wavelength.

In all experimental work conducted, a large excess of thorium nitrate was used so that, from a theoretical standpoint, the tetracycline compound in solution should be 100% complexed. However, it is apparent from Figures 2 to 8 that if any of the uncomplexed antibiotics were

FIGURE 1: MOLAR RATIO STUDY OF TETRACYCLINE-THORIUM COMPLEX SYSTEM.

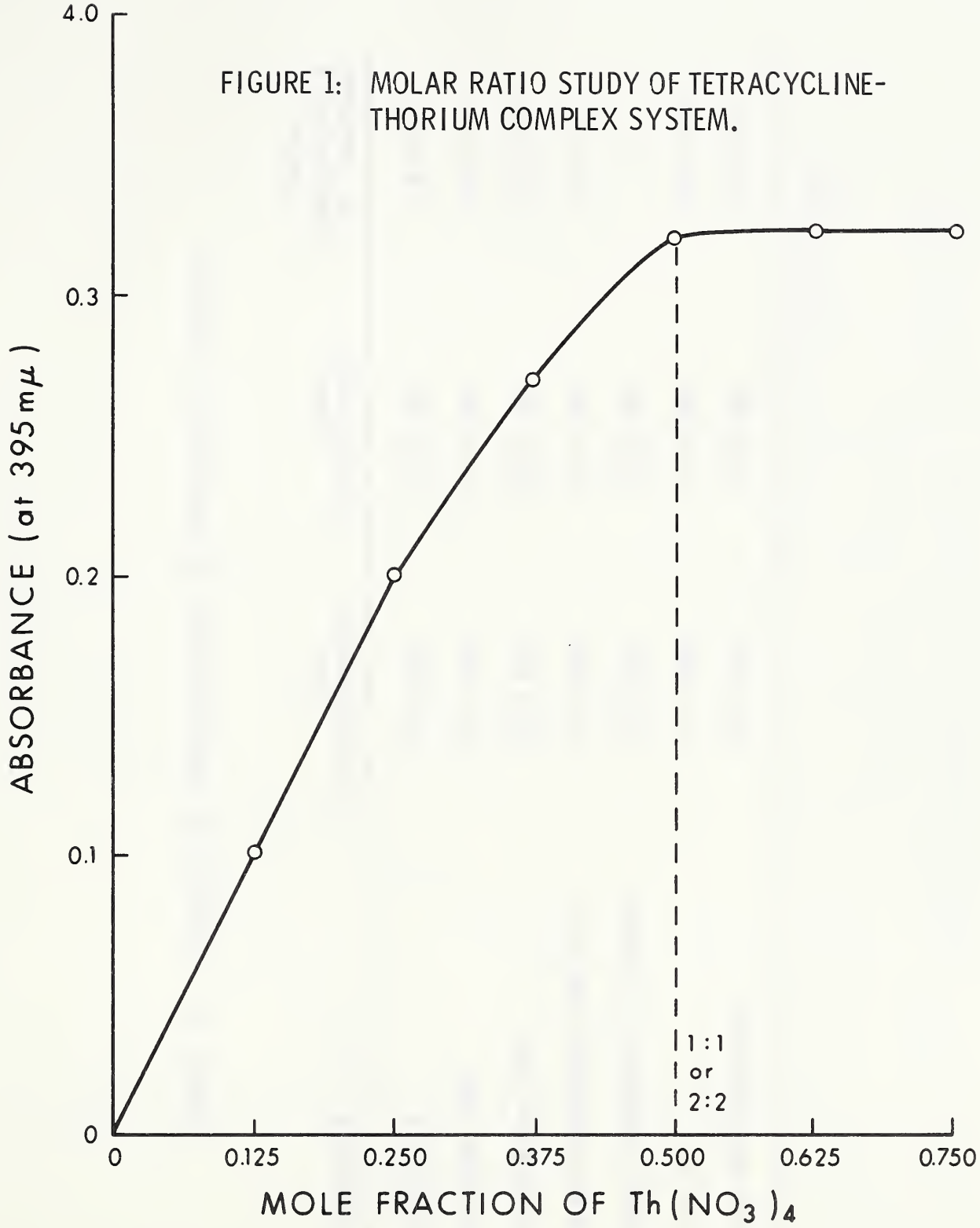


Table II. Effect of Complexation on Absorption Maxima

Compound	λmax (uncomplexed)	λmax (complexed)	Molar Absorptivity of complex
Tetracycline HCl	354 mμ	395 mμ	19,877
Oxytetracycline HCl	354 mμ	395 mμ	16,563
Chlortetracycline HCl	365 mμ	405 mμ	11,339
Demethylchlortetracycline HCl	365 mμ	405 mμ	13,702
Tetracycline phosphate complex	355 mμ	395 mμ	-
Doxycycline HCl	345 mμ	395 mμ	14,106
Doxycycline monohydrate	345 mμ	395 mμ	12,022

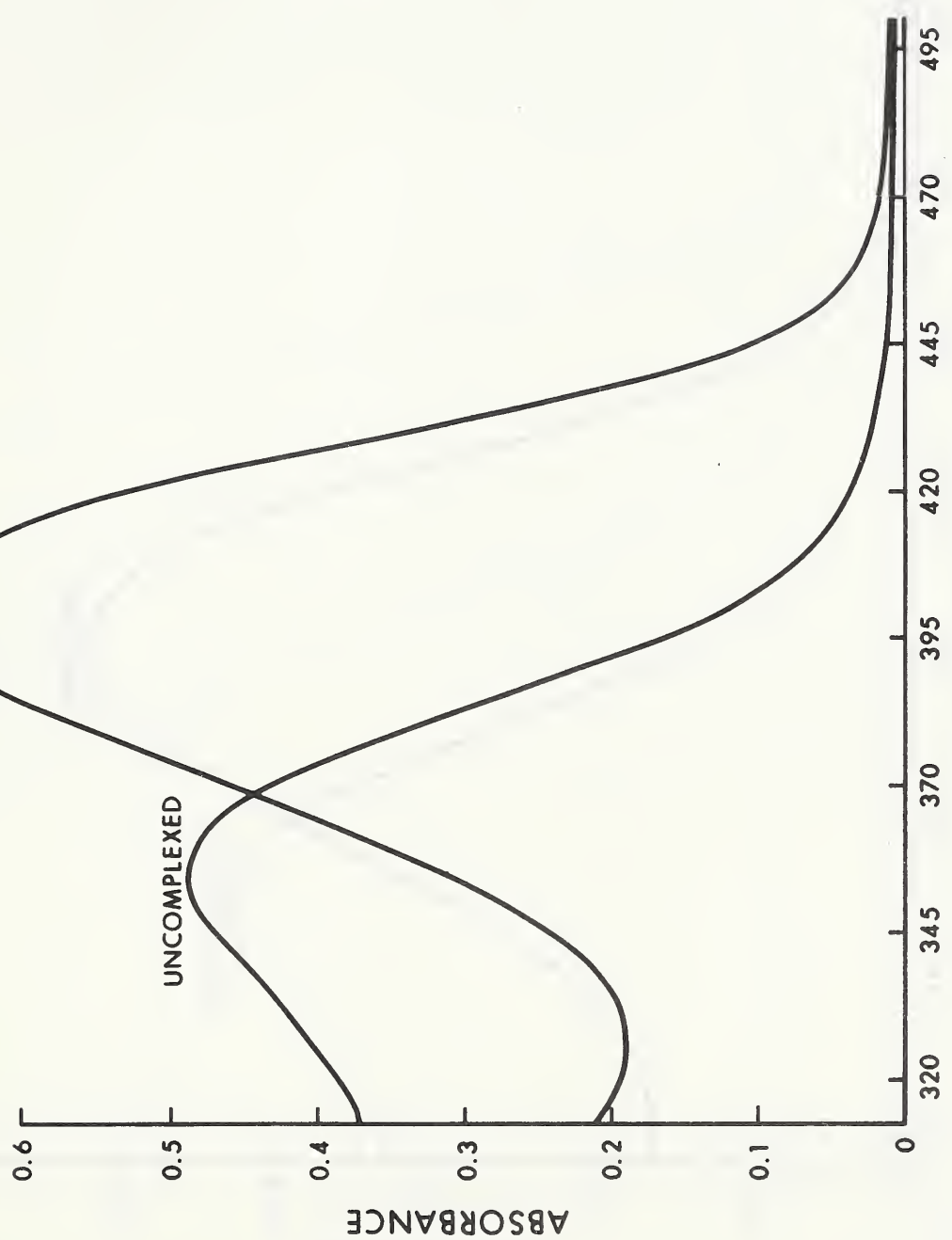


FIGURE 2: ABSORPTION SPECTRUM OF TETRACYCLINE HYDROCHLORIDE BEFORE AND AFTER COMPLEXATION WITH THORIUM.

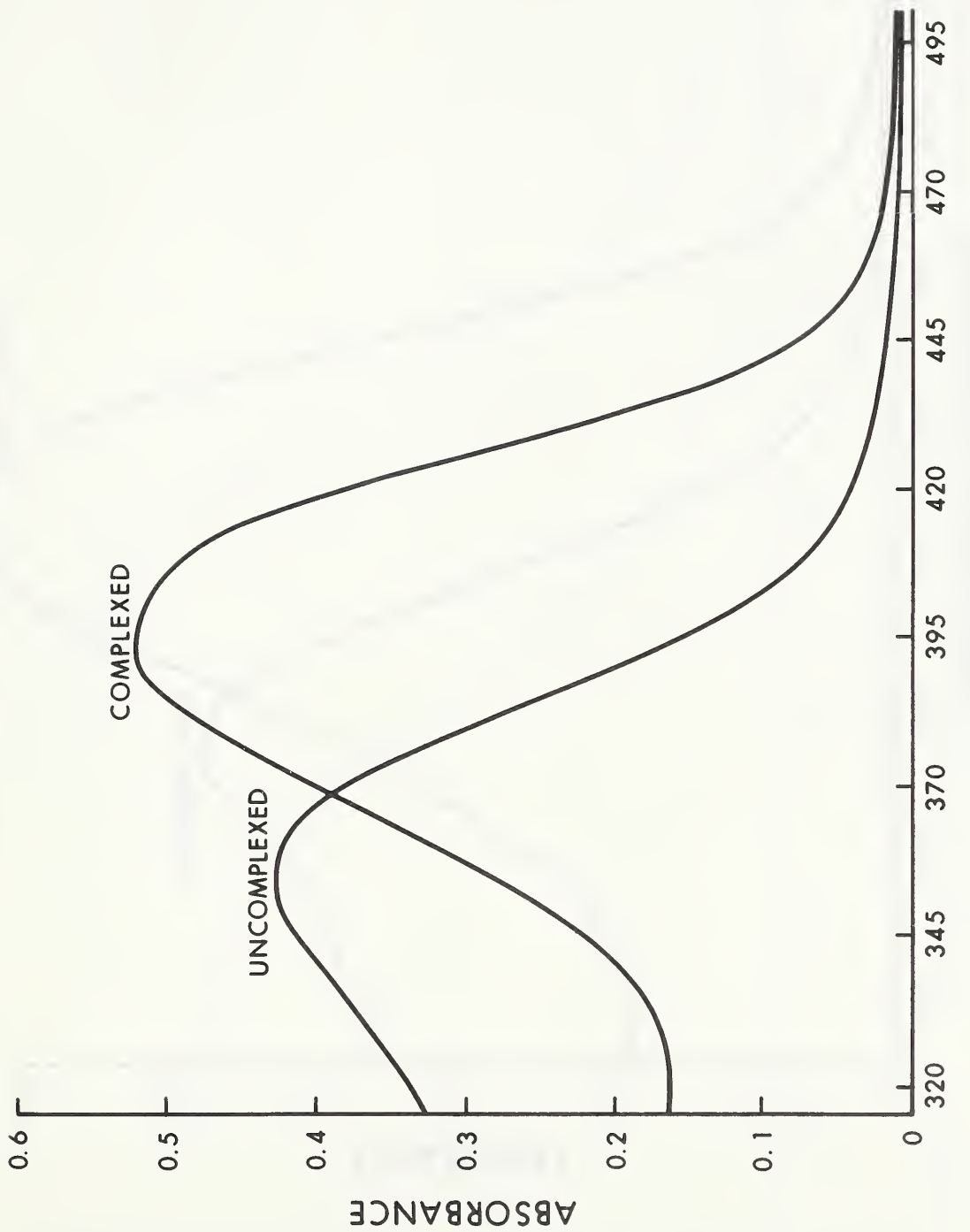


FIGURE 3: ABSORPTION SPECTRUM OF OXYTETRACYCLINE HYDROCHLORIDE BEFORE AND AFTER COMPLEXATION WITH THORIUM.

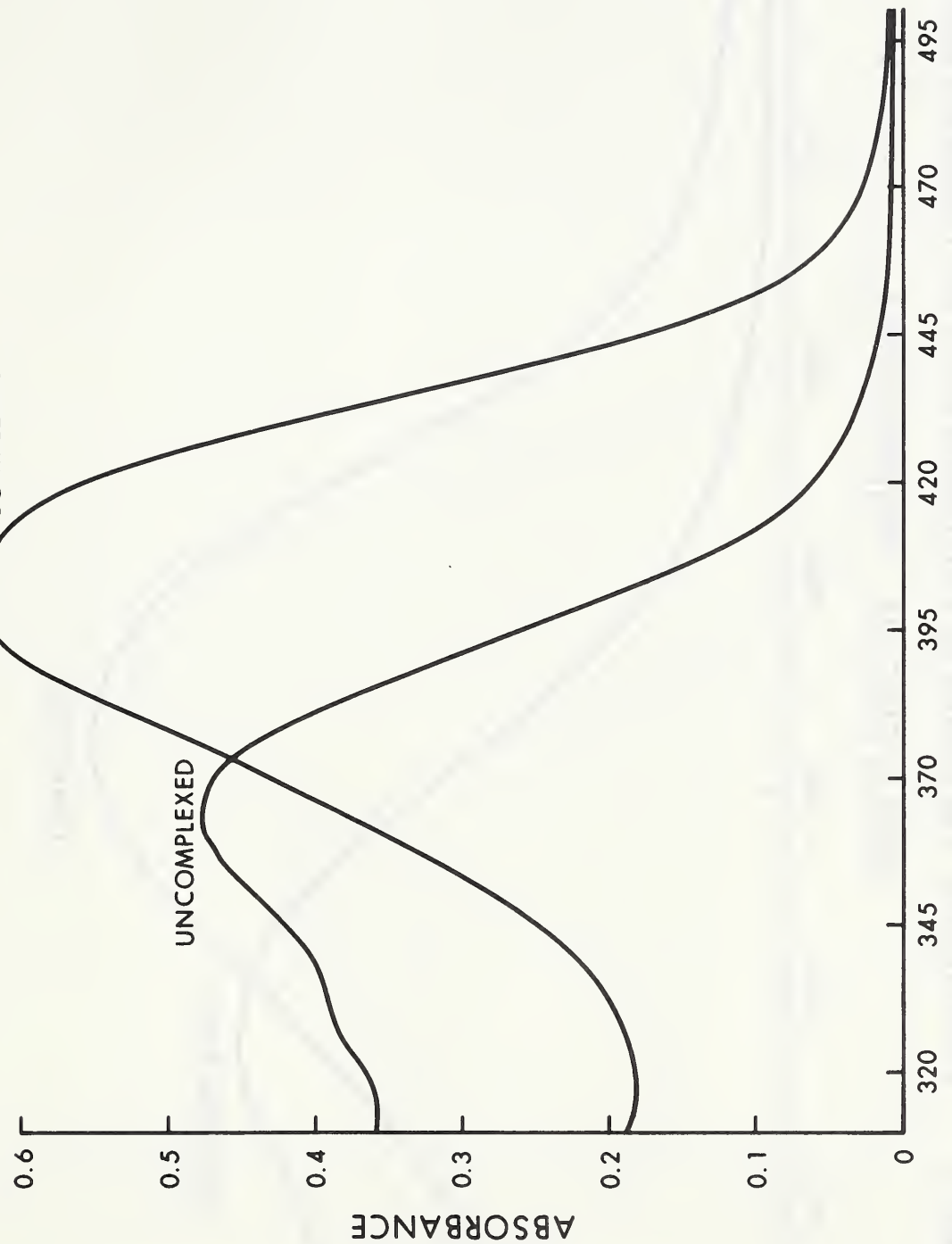


FIGURE 4: ABSORPTION SPECTRUM OF DEMETHYLCHLORTETRACYCLINE HYDROCHLORIDE BEFORE AND AFTER COMPEXATION WITH THORIUM.

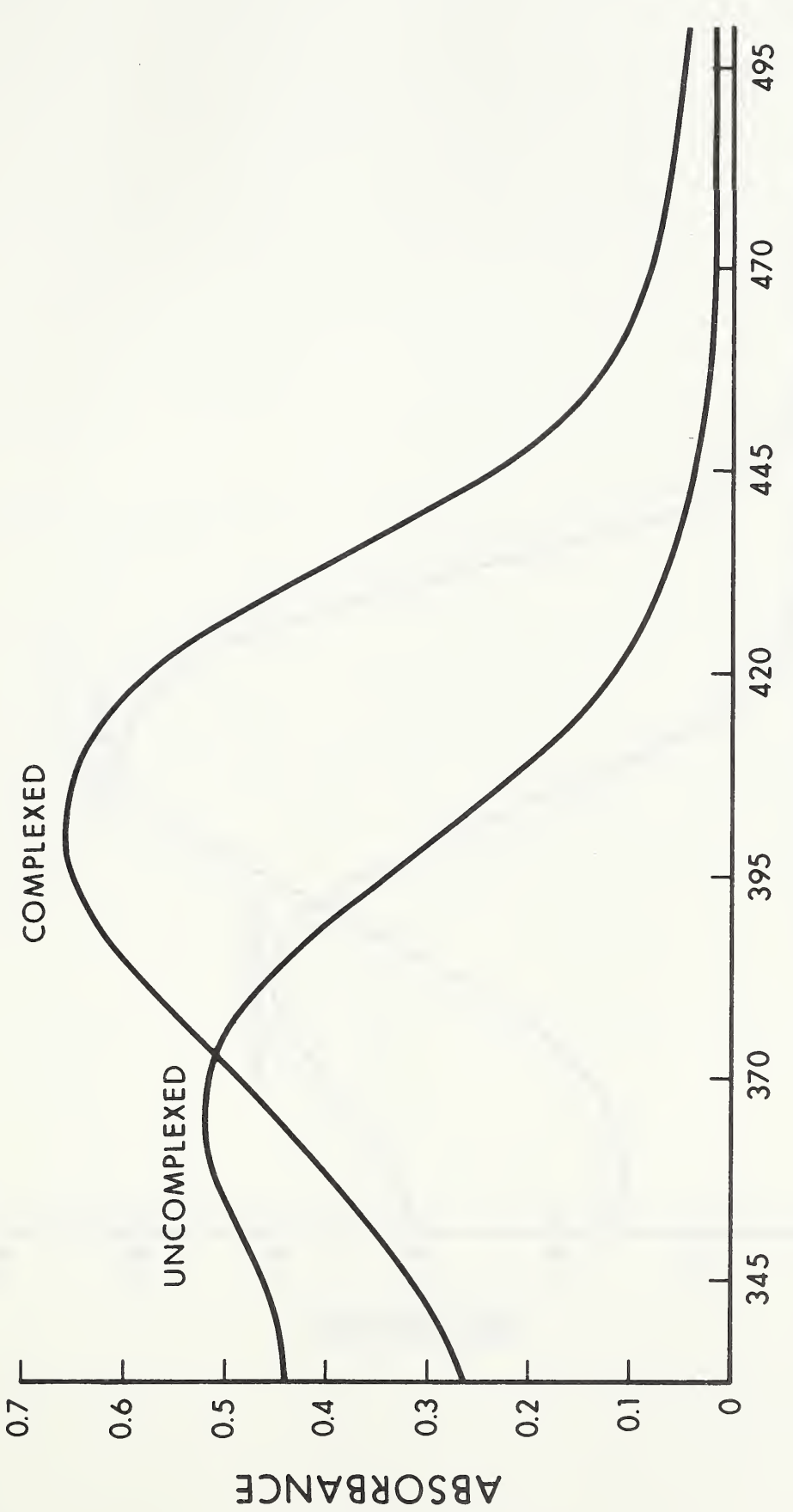


FIGURE 5: ABSORPTION SPECTRUM OF CHLORTETRACYCLINE HYDROCHLORIDE BEFORE AND AFTER COMPLEXATION WITH THORIUM.

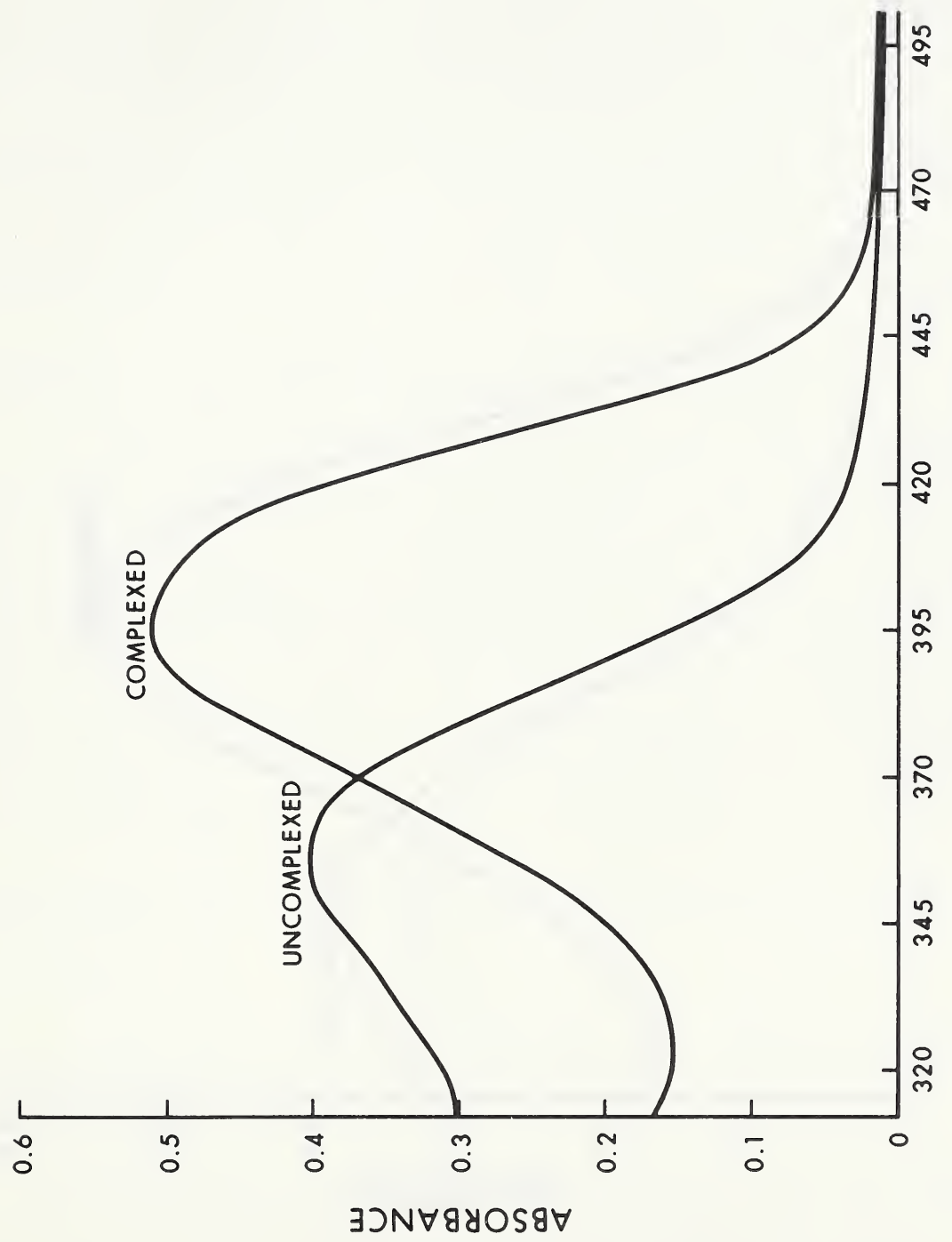


FIGURE 6: ABSORPTION SPECTRUM OF TETRACYCLINE PHOSPHATE COMPLEX BEFORE AND AFTER COMPLEXATION WITH THORIUM.

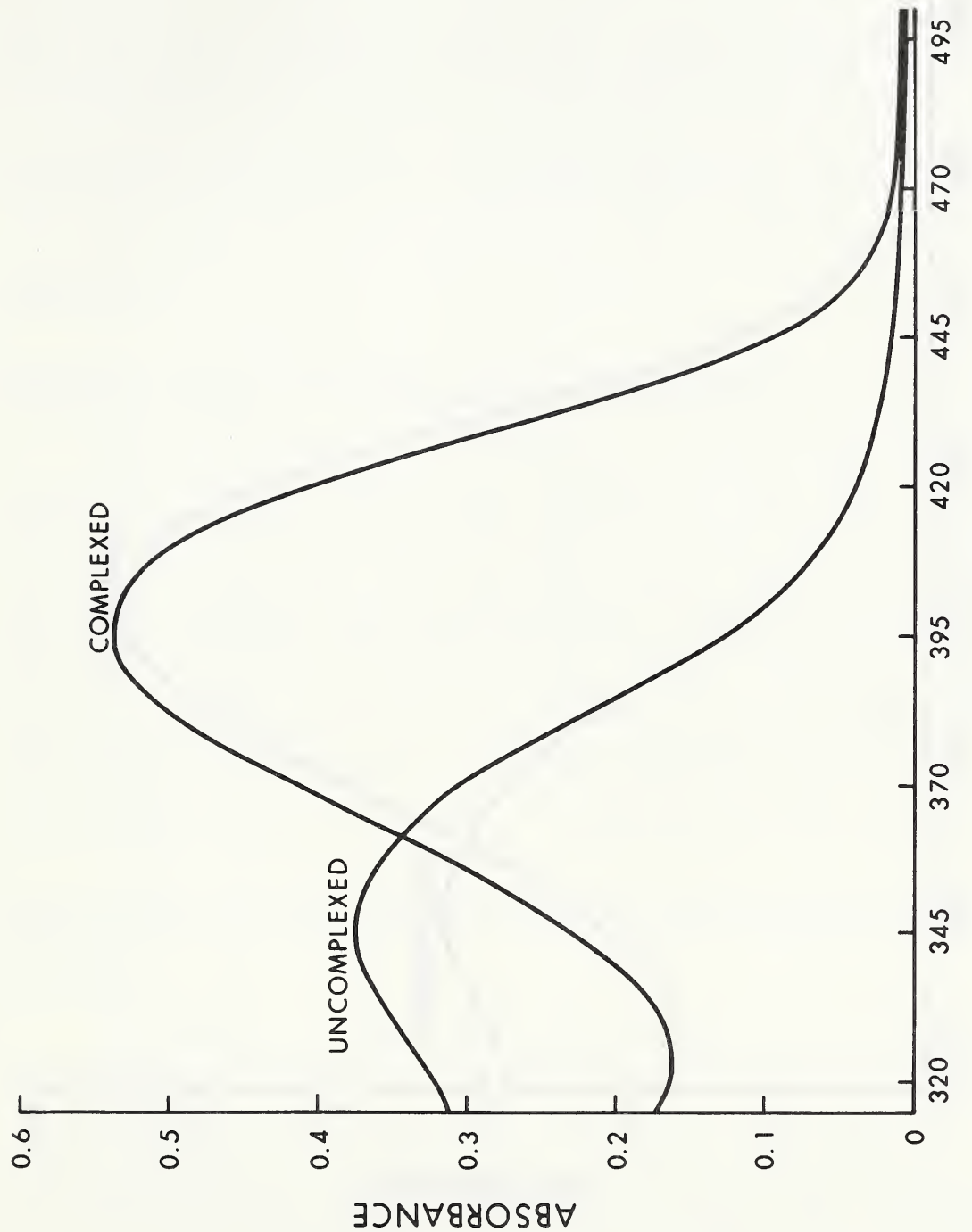


FIGURE 7: ABSORPTION SPECTRUM OF DOXYCYCLINE MONOHYDRATE BEFORE AND AFTER COMPEXATION WITH THORIUM

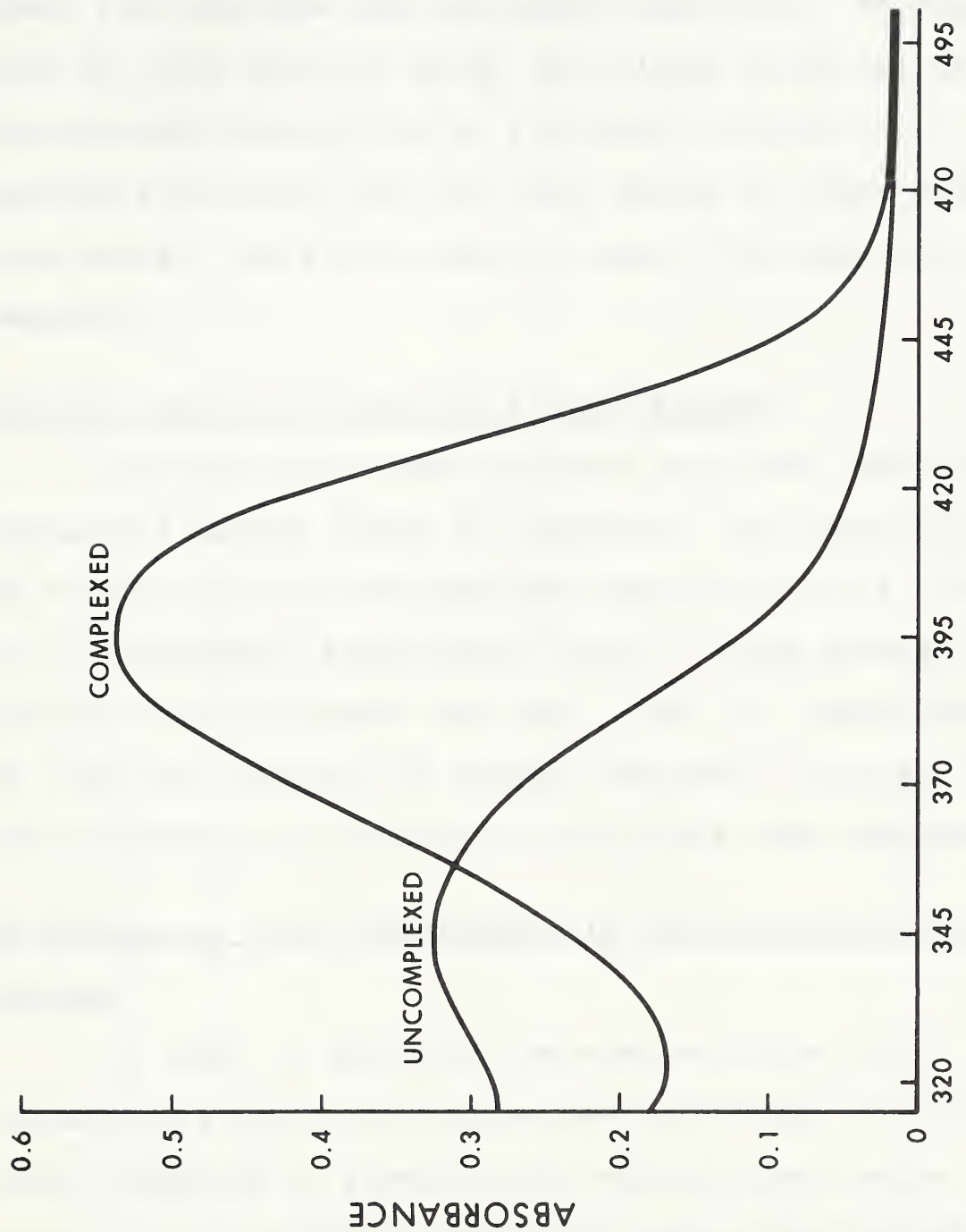


FIGURE 8: ABSORPTION SPECTRUM OF DOXYCYCLINE HYDROCHLORIDE BEFORE AND AFTER COMPEXATION WITH THORIUM.

present, a slight error would be introduced owing to a small absorbance by the uncomplexed material at the wavelength where the complexed species absorb maximally. To compensate for this possible error, all blanks contained the uncomplexed tetracycline at a suitable concentration. This procedure deviates from the usual method of blank preparation wherein the blank contains none of the substance being measured.

Optimum Quantity of Thorium Nitrate Reagent

As shown by the data obtained using the continuous variations method (Table VI, Appendix), the combining ratio of thorium with the tetracycline compounds is 1:1. Thus, in all subsequent experimental work, a large excess of thorium nitrate reagent was used. That is, approximately 10 times the quantity of reagent required to achieve a 1:1 stoichiometry was utilized to facilitate 100% complexation.

pH Effects on Color Development in Tetracycline-Thorium Systems

In order to determine the most suitable pH for color formation, a series of studies was undertaken in which the color intensity of a particular tetracycline-thorium complex was recorded at various pH values over an extended period of time. Thus, it was possible to assess the color developed at each pH value in terms of its intensity and stability, and to subsequently select a working pH which would permit the highest accuracy in colorimetric

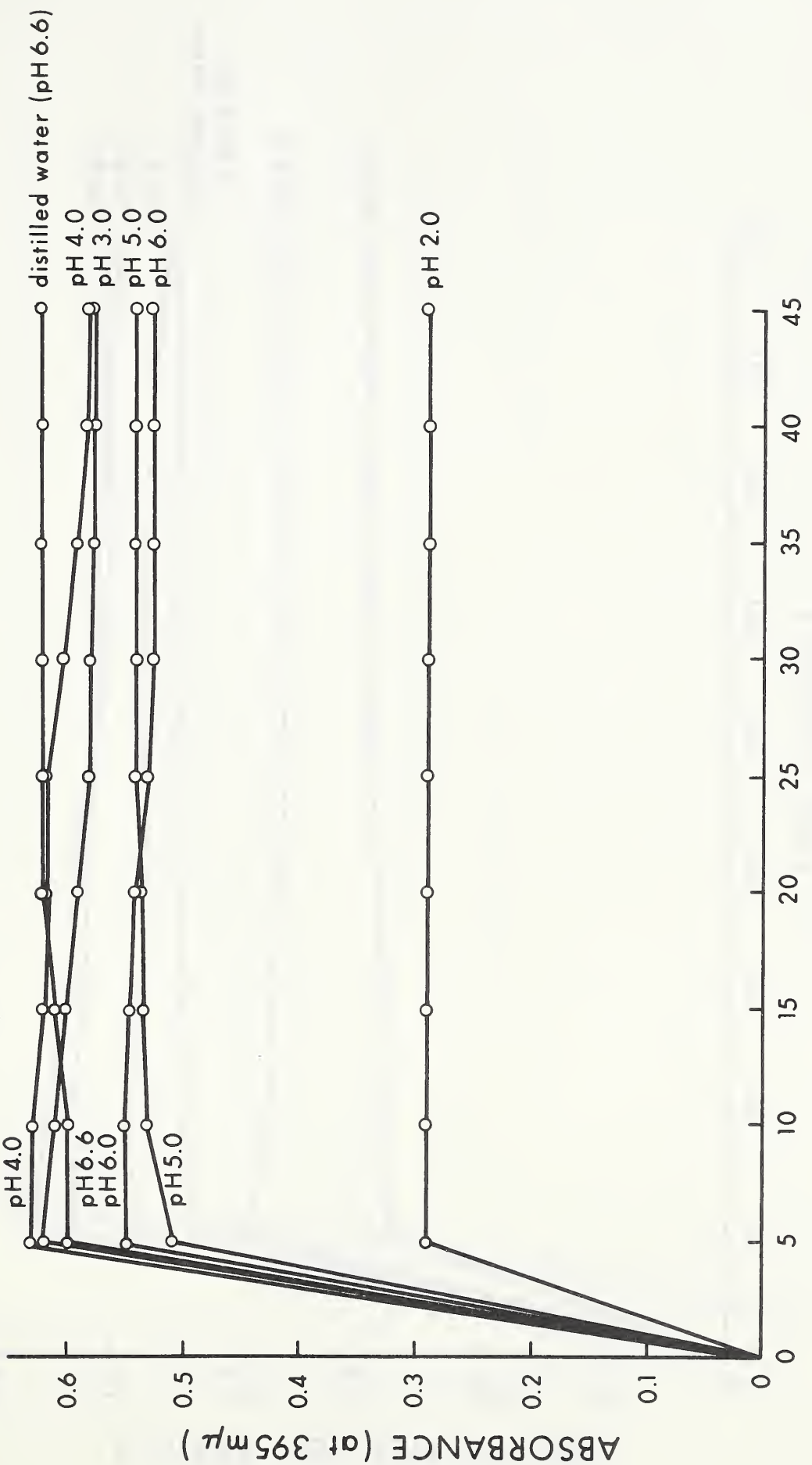


FIGURE 9: EFFECT OF pH ON COLOR DEVELOPMENT FOR TETRACYCLINE HCl.

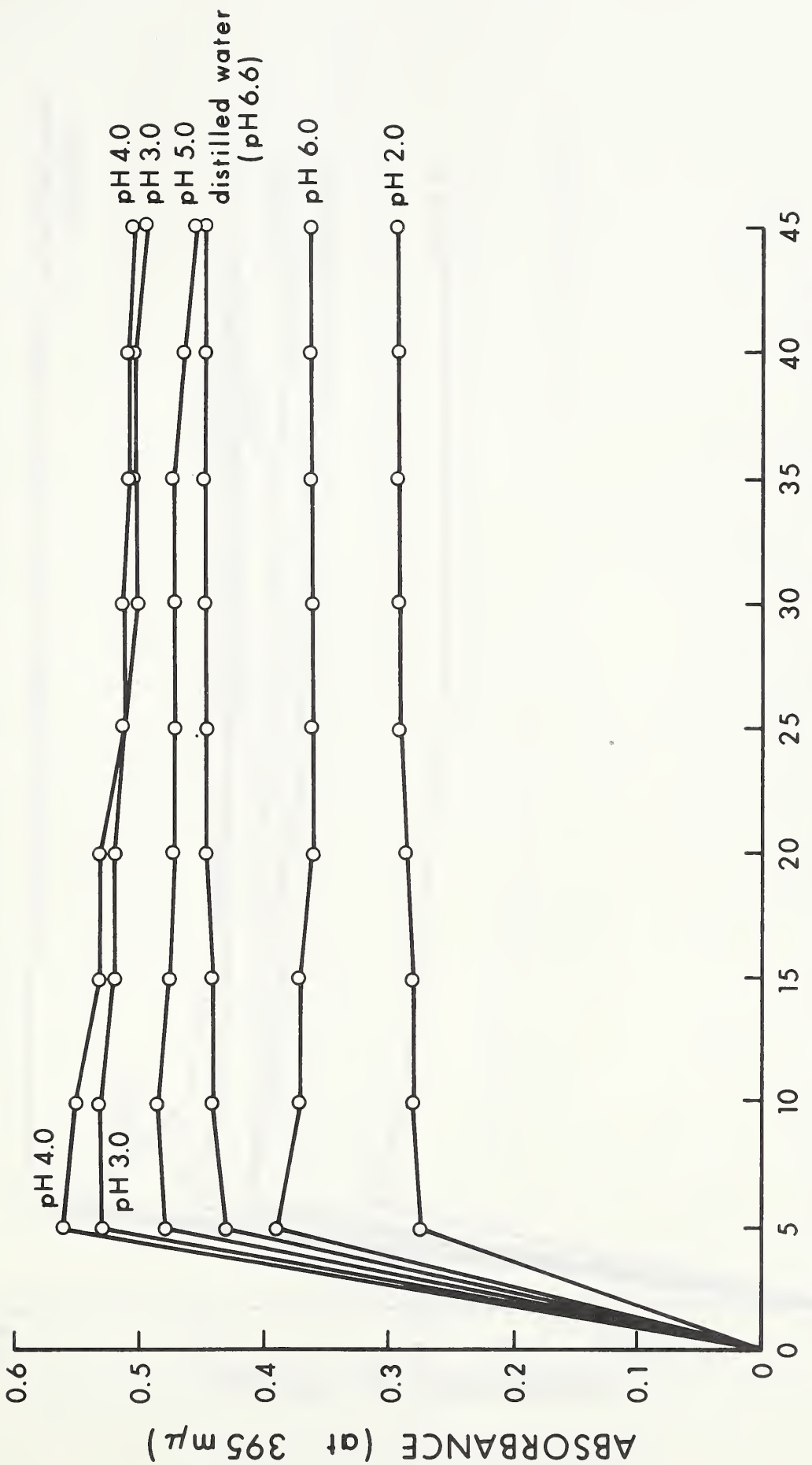


FIGURE 10: EFFECT OF pH ON COLOR DEVELOPMENT FOR OXYTETRACYCLINE HCl.

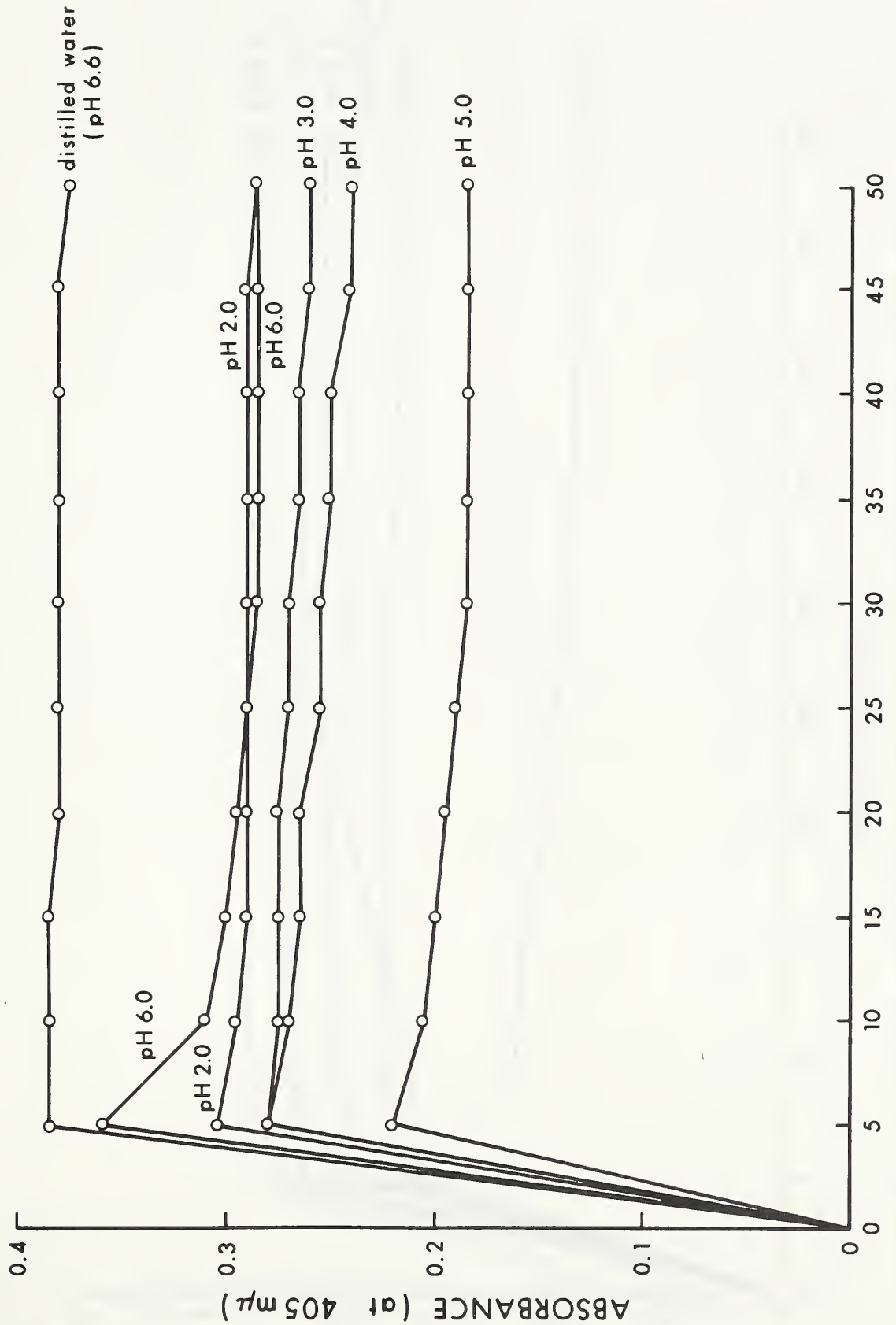


FIGURE 11: EFFECT OF pH ON COLOR DEVELOPMENT FOR DEMETHYLCHLORTETRACYCLINE HCl.

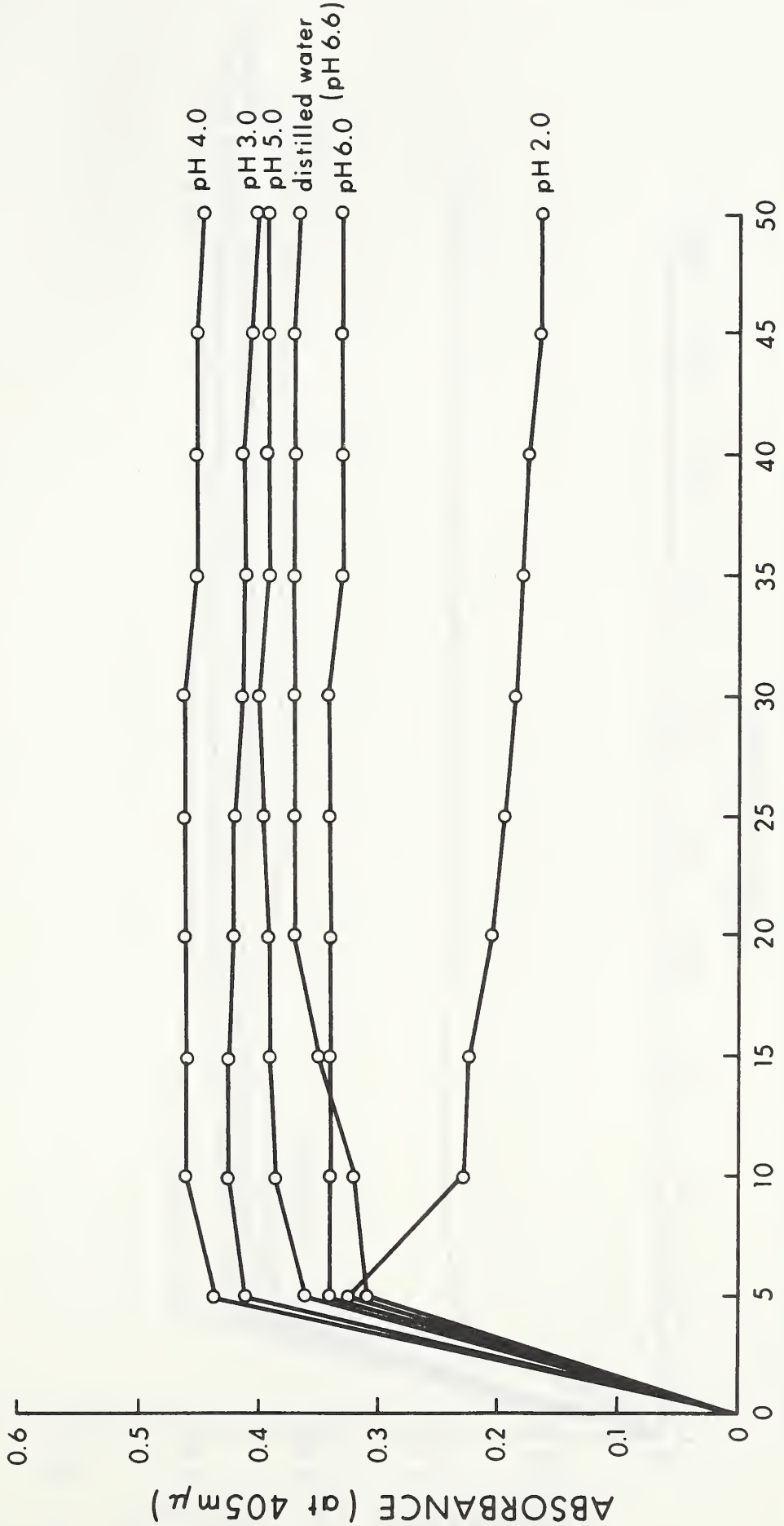


FIGURE 12: EFFECT OF pH ON COLOR DEVELOPMENT FOR CHLORTETRACYCLINE HCl.

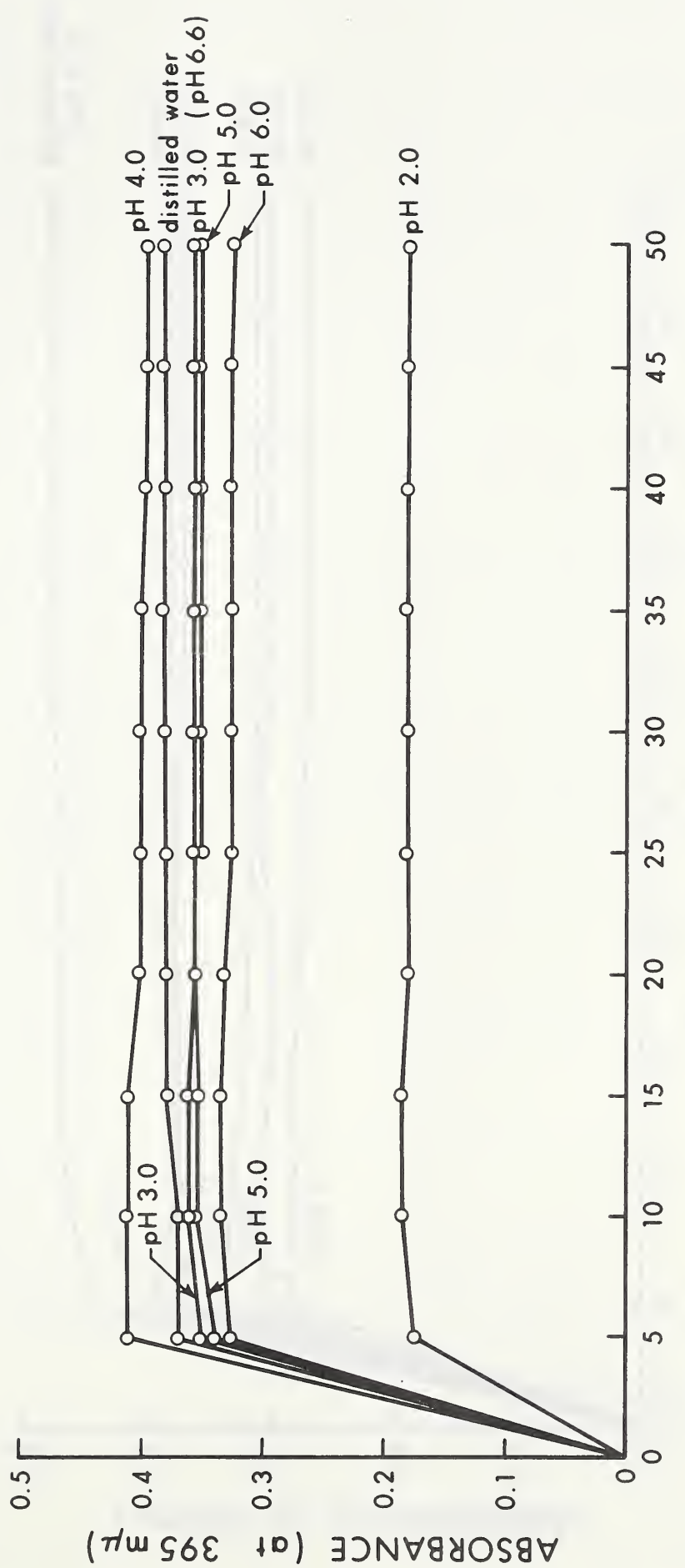


FIGURE 13: EFFECT OF pH ON COLOR DEVELOPMENT FOR TETRACYCLINE PHOSPHATE COMPLEX.

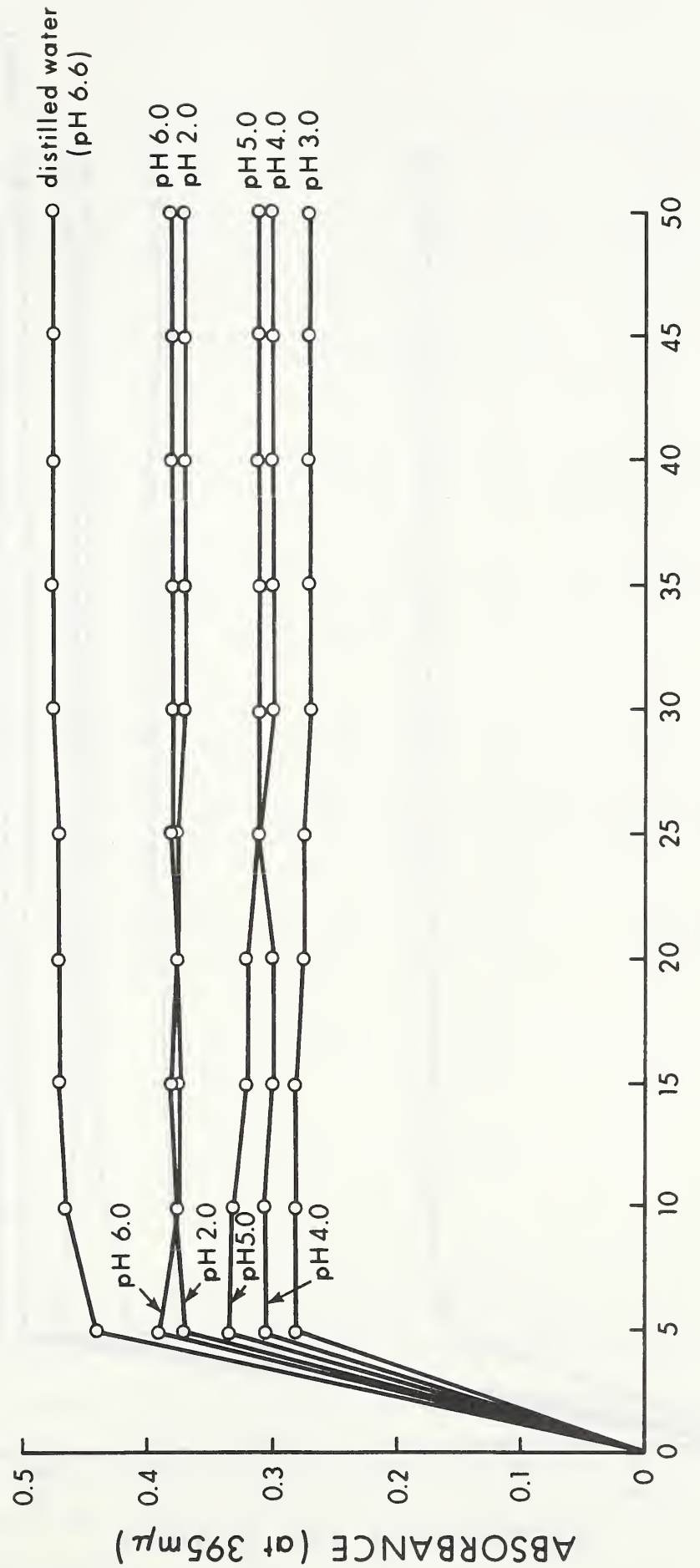


FIGURE 14: EFFECT OF pH ON COLOR DEVELOPMENT FOR DOXYCYCLINE MONOHYDRATE.

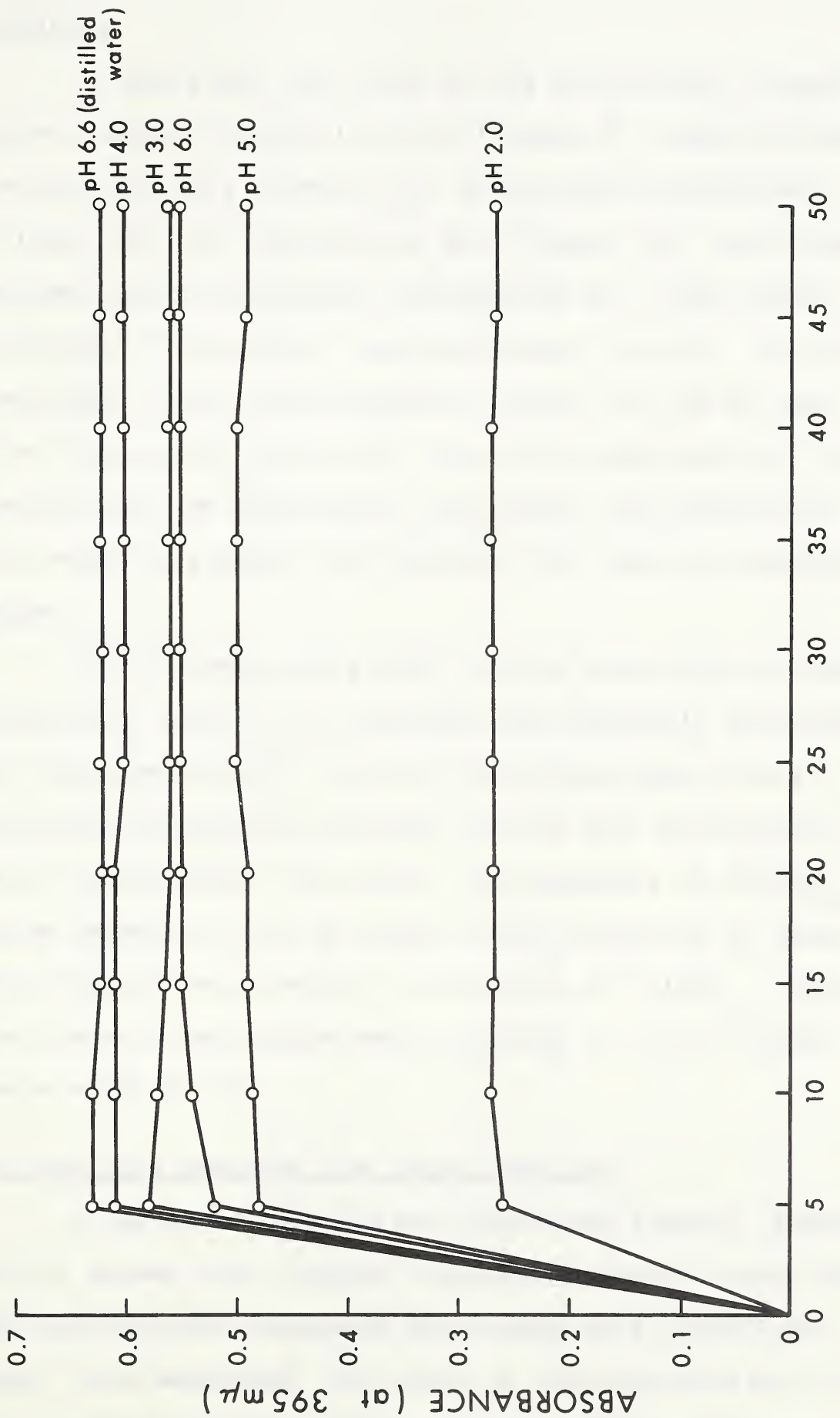


FIGURE 15: EFFECT OF pH ON COLOR DEVELOPMENT FOR DOXYCYCLINE HCl.

readings.

It was found that four of the antibiotics investigated, namely tetracycline HCl (Figure 9), demethylchlortetracycline HCl (Figure 11), doxycycline monohydrate (Figure 14), and doxycycline HCl (Figure 15), exhibited maximal color development accompanied by a high degree of stability at the pH of distilled water (~6.6). For the remainder of the tetracyclines studied, it can be seen from Figures 10, 12 and 13, that while optimum color intensity was not achieved at this point, the stability of the color at a pH of 6.6 justifies its use as a working value.

When attempts were made to take quantitative readings above a pH of 7.0, results were adversely affected by the appearance of a white, flocculent precipitate. Although complexation between thorium and tetracycline still occurs above this point, the presence of $Th(NO_3)_4$ in large excess at this pH leads to the formation of insoluble hydroxides primarily in the form of $Th(OH)_4$. Thus the present spectrophotometric method is of no utility above a pH of 7.0.

Minimum Time Required for Color Stability

A series of time versus absorbance studies (Figures 16-22) showed that colored complexes between thorium and the tetracycline compounds are formed very rapidly and that, once developed, the color is characterized by a high degree of stability. The intensities of the colors were

Table III. Suitable pH Range and Minimum Time for Stable Color Formation

Compound	λmax (a) (complexed)	Minimum Time (minutes)	Suitable pH Range
Tetracycline HCl	395 mμ	20	3-7
Oxytetracycline HCl	395 mμ	20	6.0-6.6
Chlortetracycline HCl	405 mμ	20	3-7
Demethylchlortetracycline HCl	405 mμ	20	6.0-6.6
Tetracycline Phosphate Complex	395 mμ	20	3-7
Doxycycline HCl	395 mμ	35	3-7
Doxycycline monohydrate	395 mμ	35	6.6

(a) All λmax obtained at pH 6.6.

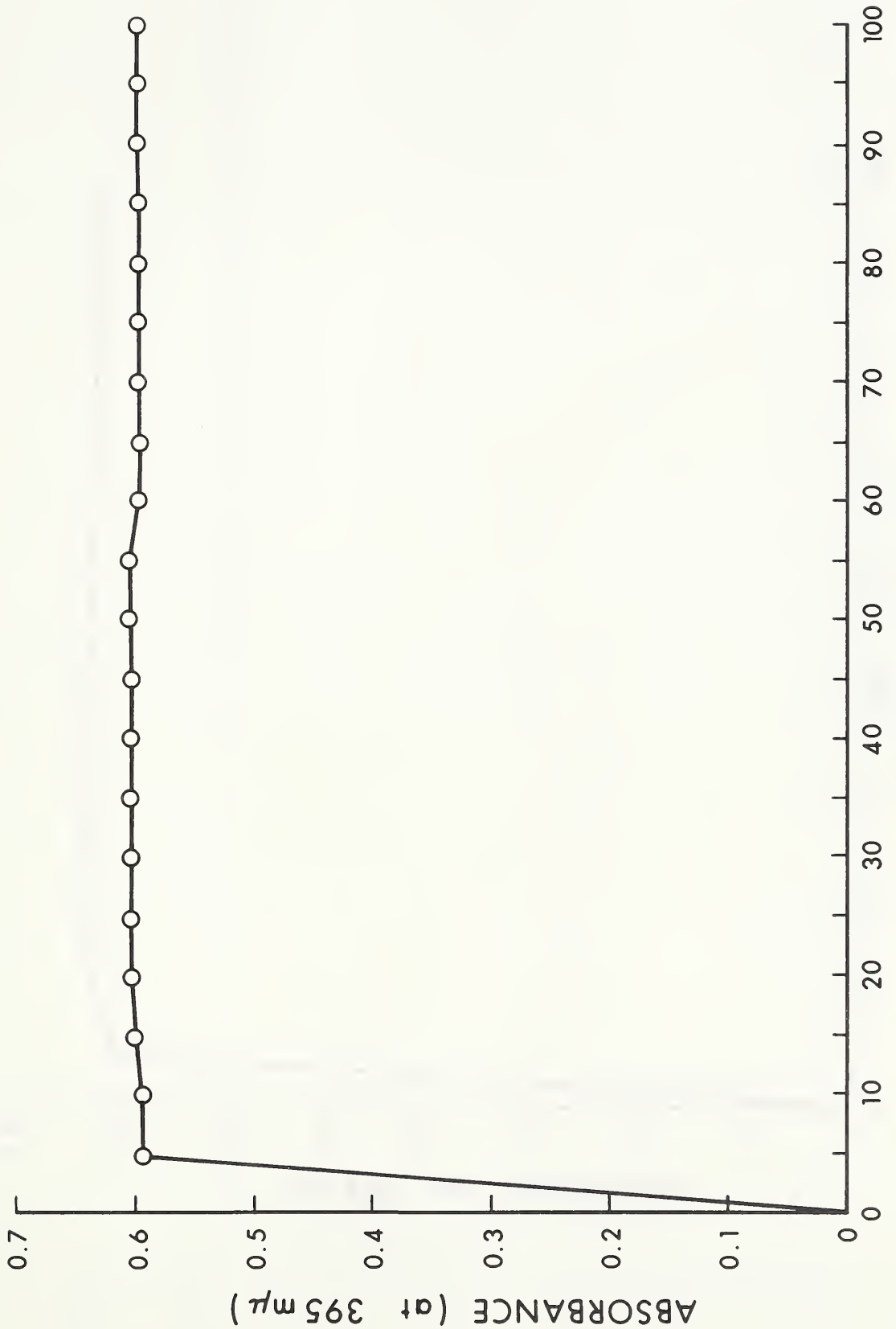


FIGURE 16: EFFECT OF TIME ON COLOR DEVELOPMENT FOR TETRACYCLINE HCl.

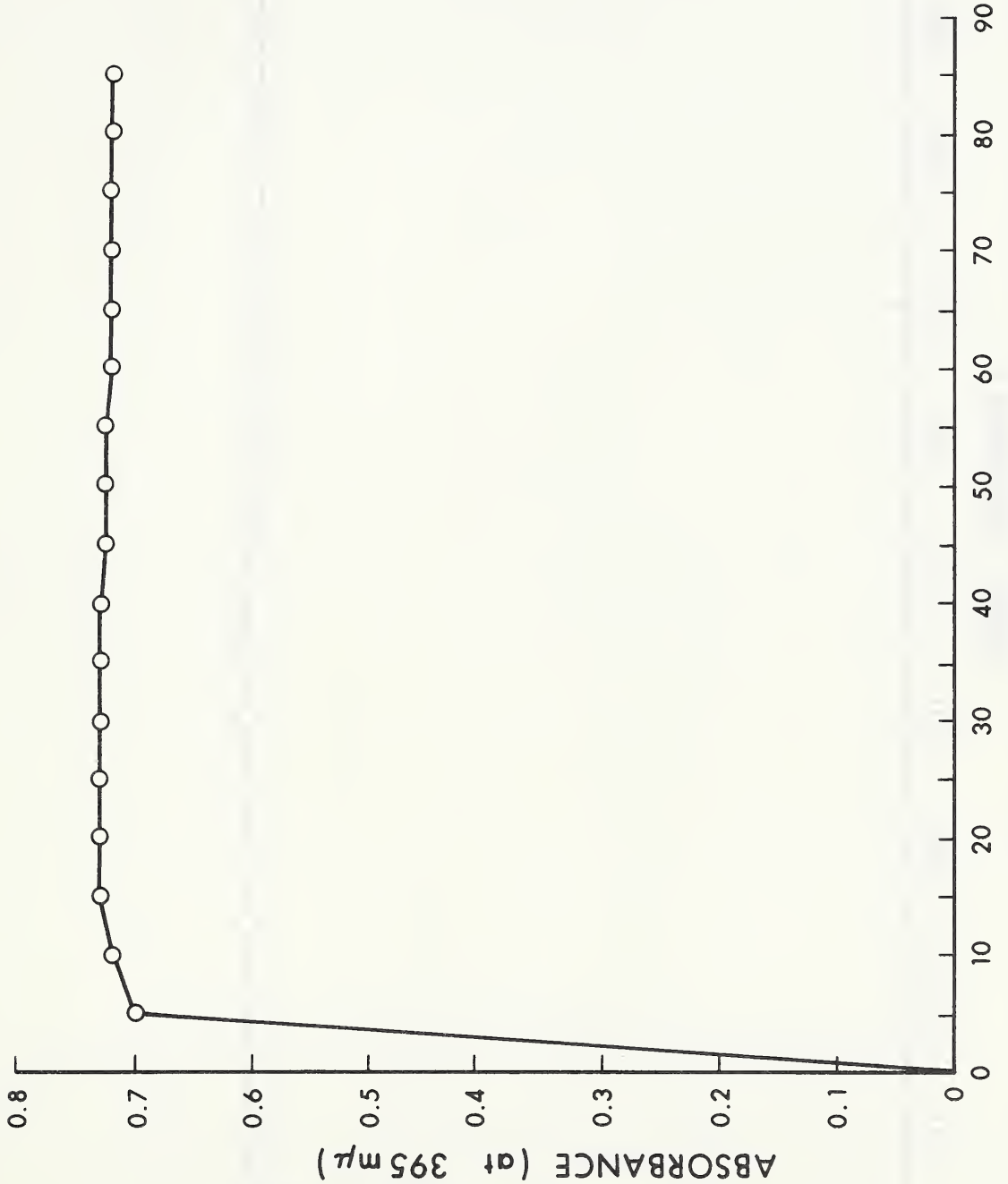


FIGURE 17: EFFECT OF TIME ON COLOR DEVELOPMENT FOR OXYTETRACYCLINE HCl.

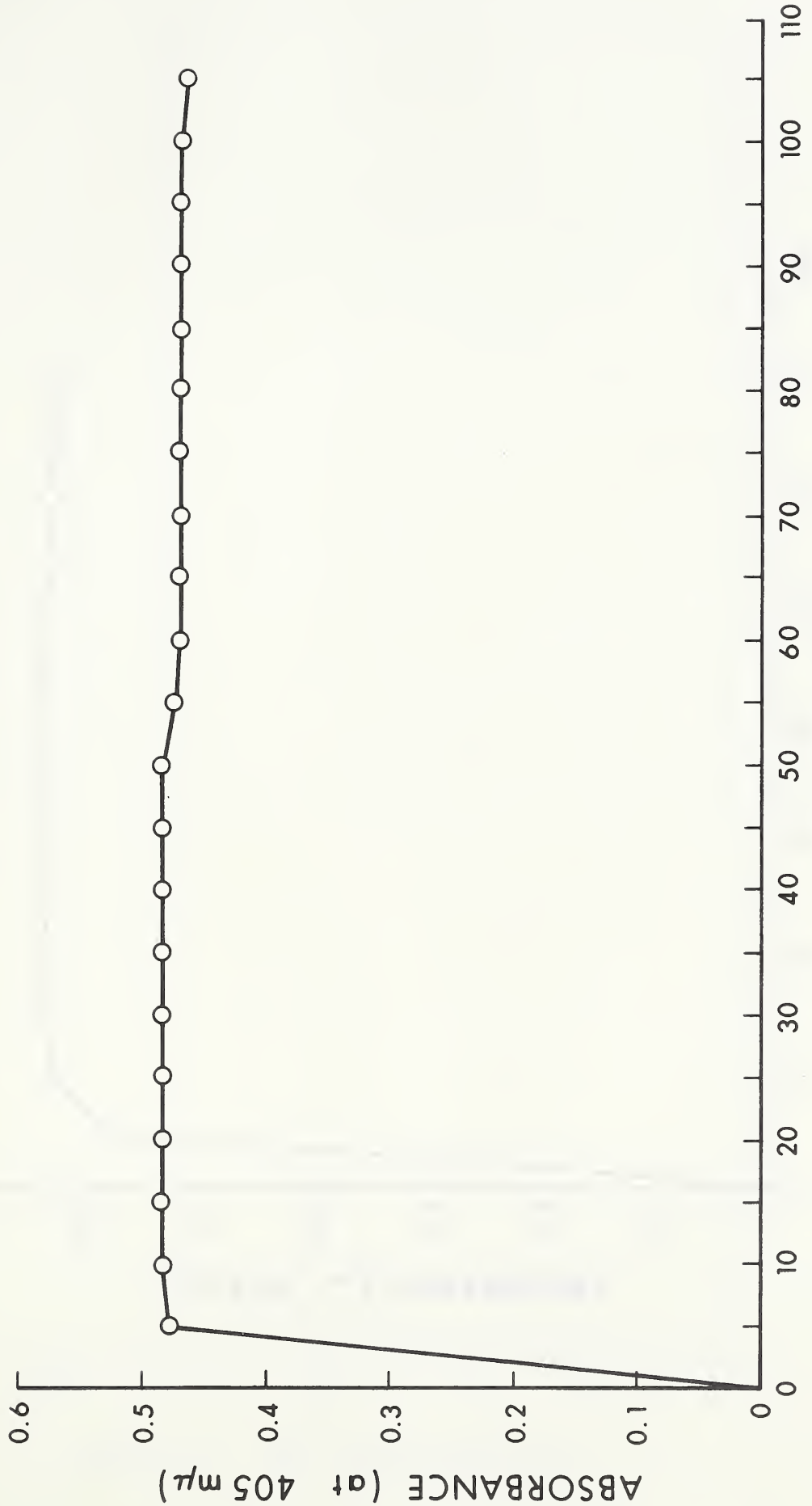


FIGURE 18: EFFECT OF TIME ON COLOR DEVELOPMENT FOR DEMETHYLCHLORTETRACYCLINE HCl.

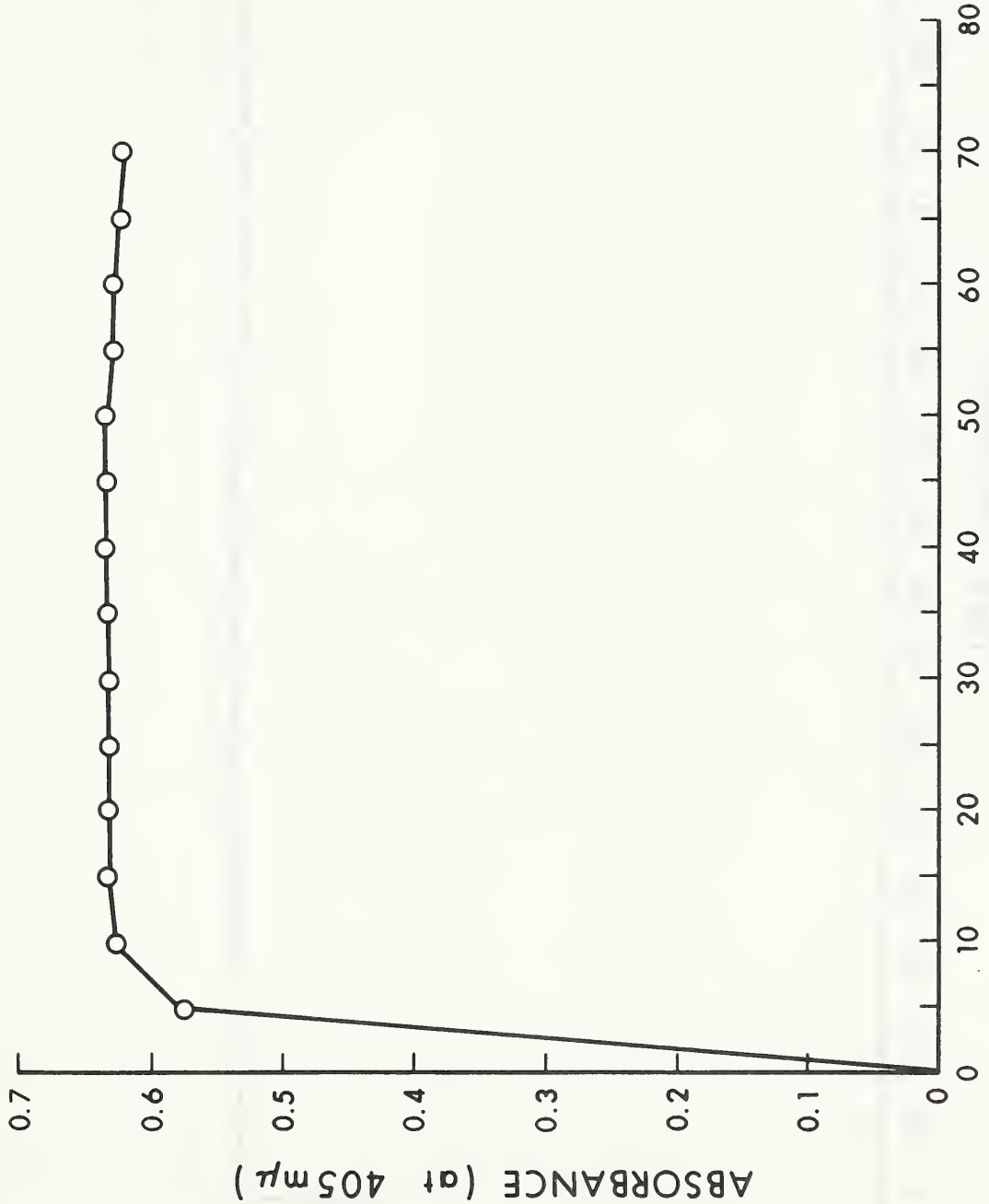


FIGURE 19: EFFECT OF TIME ON COLOR DEVELOPMENT FOR CHLORTETRACYCLINE HCl.

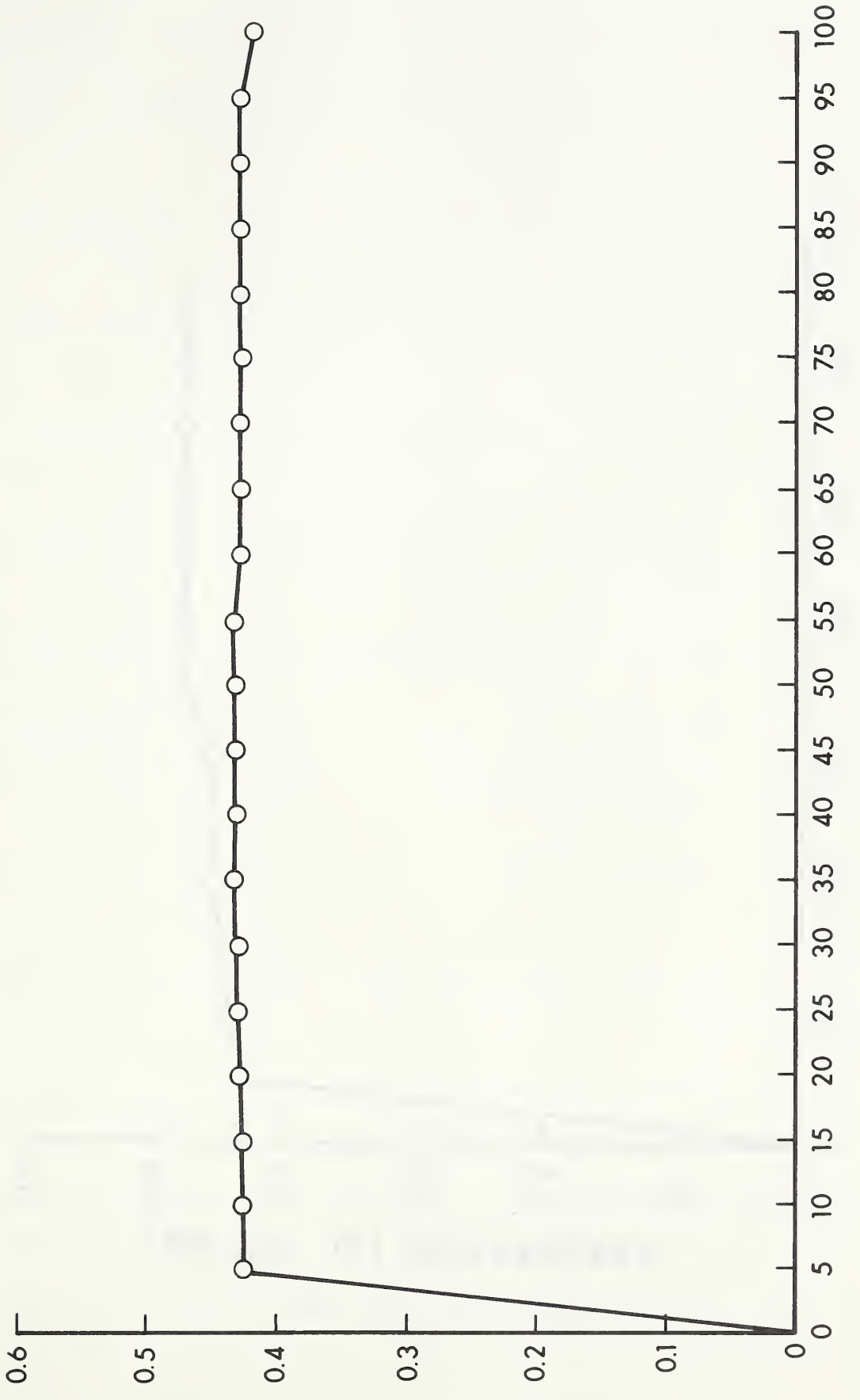


FIGURE 20: EFFECT OF TIME ON COLOR DEVELOPMENT FOR TETRACYCLINE PHOSPHATE COMPLEX.

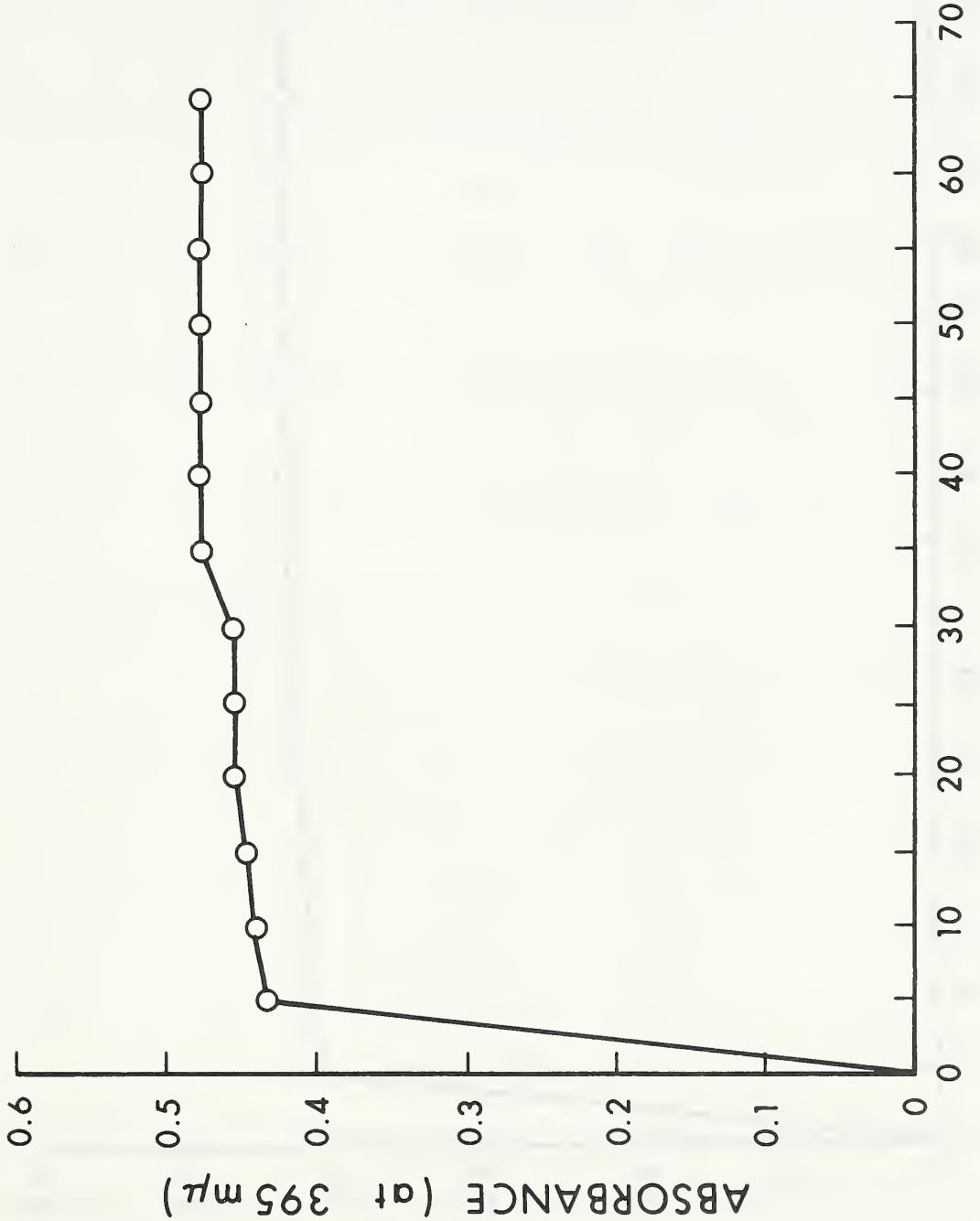


FIGURE 21: EFFECT OF TIME ON COLOR DEVELOPMENT FOR DOXYCYCLINE MONOHYDRATE.

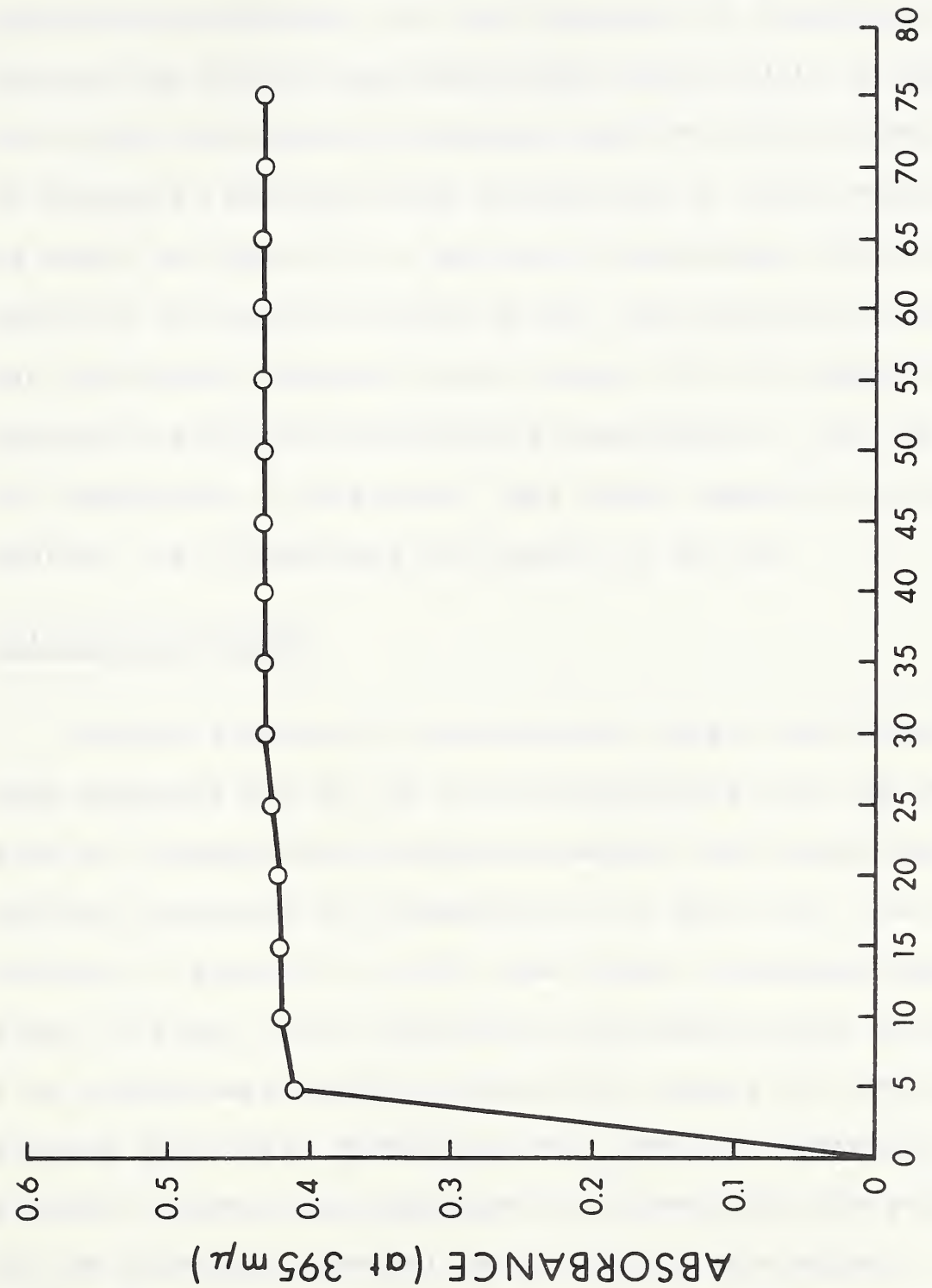


FIGURE 22: EFFECT OF TIME ON COLOR DEVELOPMENT FOR DOXYCYCLINE HCl.

subject to a few very slight fluctuations within a period of up to 75 minutes following the initial combining of $Th(NO_3)_4$ with the antibiotics. In all instances an absorbance plateau was reached and maintained within 20 to 35 minutes such that satisfactory readings could be taken without loss of accuracy resulting from alterations in color stability. As shown in Table III, a period of 20 minutes after the addition of thorium nitrate to the tetracycline solutions was considered adequate in all cases with the exception of doxycycline HCl and doxycycline monohydrate. For the latter two compounds, a 35 minute time lapse appeared to be essential, as illustrated by Figures 21 and 22.

Calibration Curves

Standard plots of concentration versus absorbance were prepared for all of the tetracyclines with the exception of tetracycline phosphate complex and doxycycline hyclate according to Procedures E (1) and E (2). As illustrated in Figures 23 to 31, good linear relationships were found to exist in all instances and reproducible calibration curves were readily obtained by taking the average results from three determinations (Table X, Appendix). All standard curves were corrected to account for the purity of the reference material as previously determined.

In the preparation of a calibration curve for tetracycline phosphate complex, Procedure E (2) was also employed. However, since the actual composition of the complex

is variable and may in fact alter from batch to batch, a statement regarding concentration of the complex cannot give a true indication of the tetracycline concentration. For this reason, a comparison was made between absorbance readings obtained using a particular concentration of tetracycline phosphate complex and those obtained with the same concentration of tetracycline base. This data is shown in Table XII of the Appendix and it can be seen that a correlation between concentration of the tetracycline phosphate complex and the equivalent concentration of tetracycline has been determined. In the present investigation, it has been shown that 1 gram of the complex material is equivalent to 0.678 gram of tetracycline base. Thus an absorbance versus concentration curve has been prepared for tetracycline phosphate complex (Figure 28), but all readings of concentration taken from this curve were subsequently multiplied by a factor of 0.678 to obtain a determination of the equivalent tetracycline content. It must be noted that a new factor must be similarly determined for every new batch of tetracycline phosphate complex.

Another difficulty arose in the preparation of a calibration curve for doxycycline hyclate. Since no doxycycline hyclate reference material was available, it was necessary to prepare a standard absorbance versus concentration curve for doxycycline hydrochloride (Figure 30) and subsequently to extrapolate this data in such a way that it theoretically pertained to doxycycline hyclate in

FIGURE 23: CALIBRATION CURVE FOR TETRACYCLINE

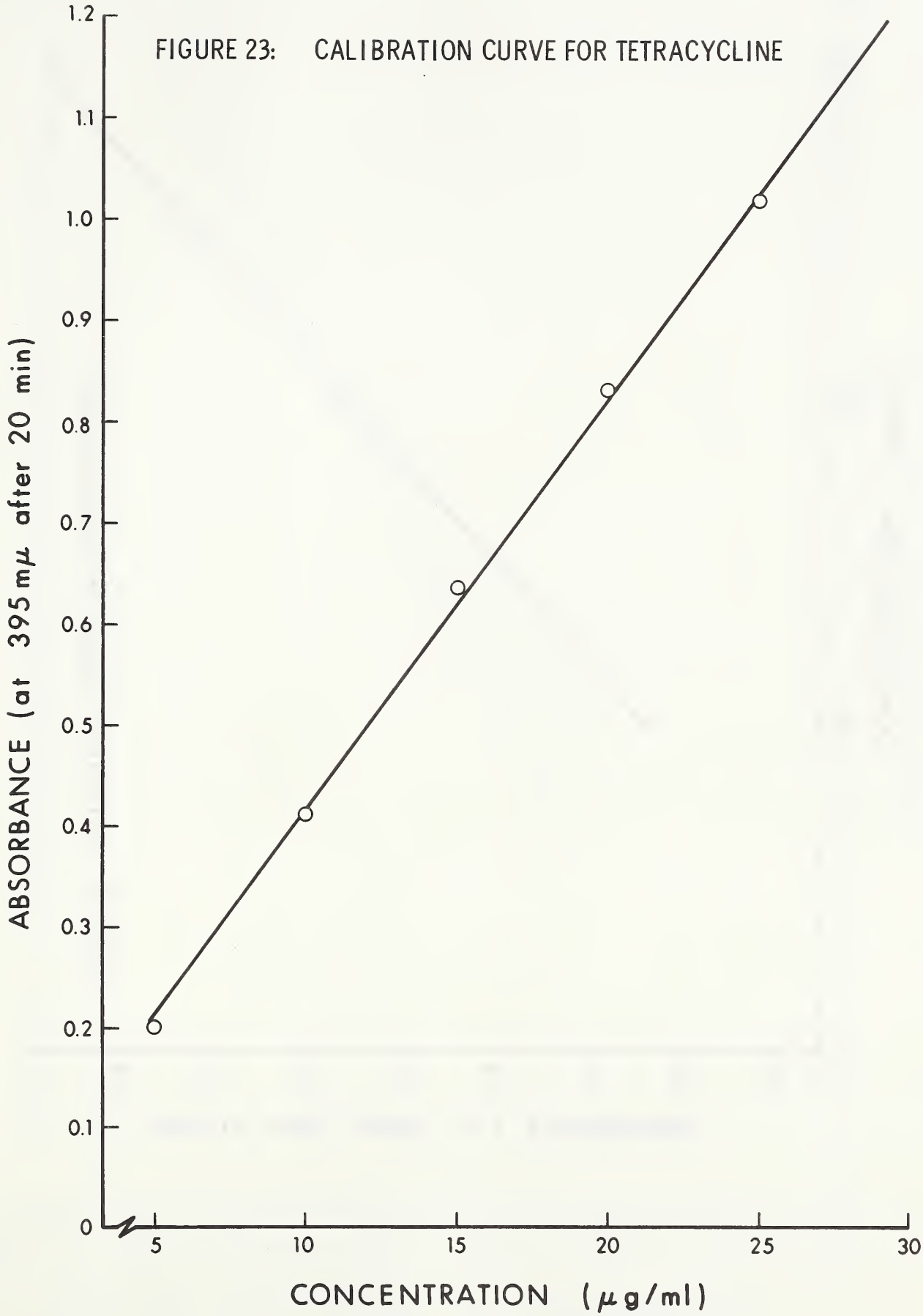


FIGURE 24: CALIBRATION CURVE FOR OXYTETRACYCLINE.

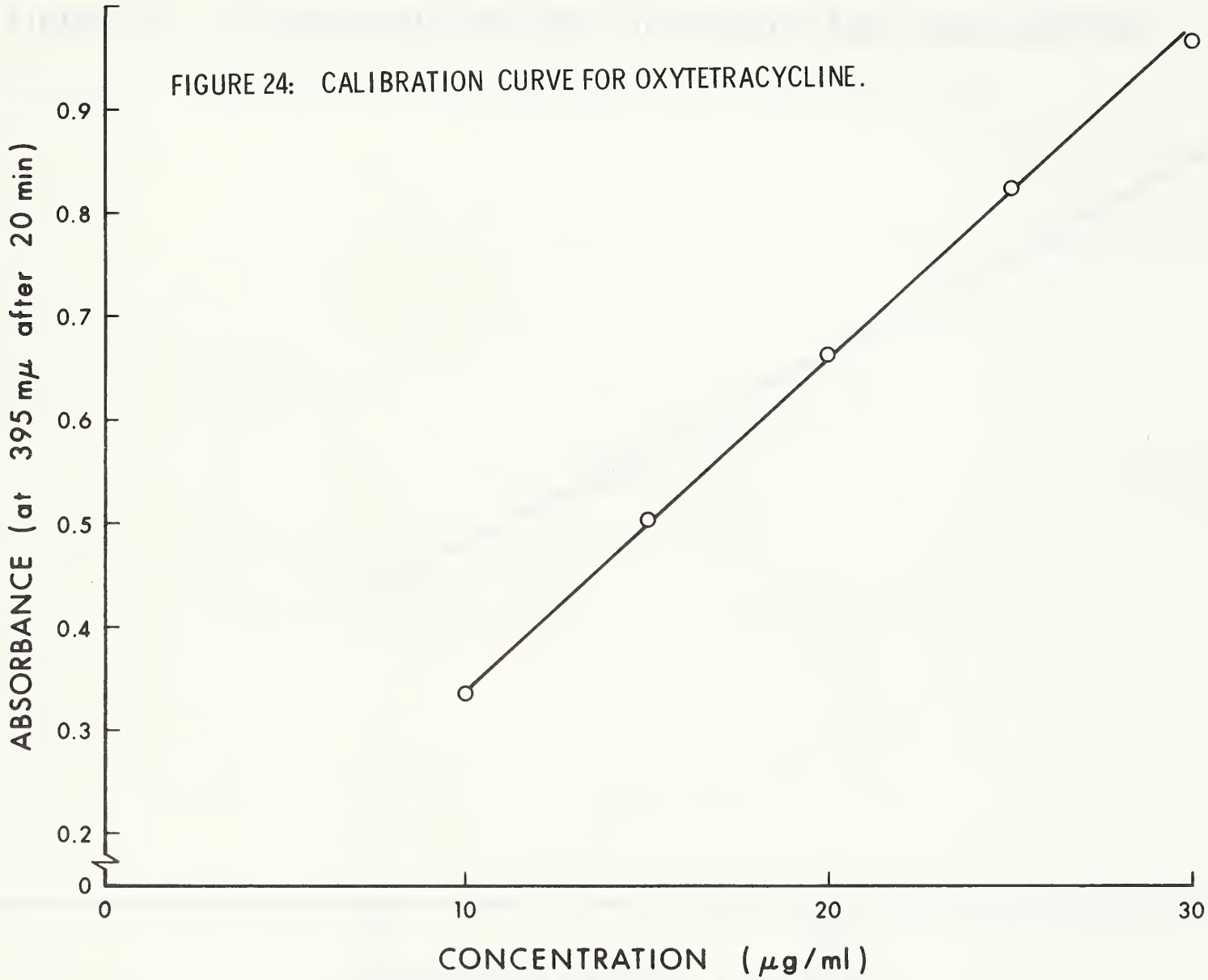


FIGURE 25: CALIBRATION CURVE FOR OXYTETRACYCLINE IN CHLOROFORM

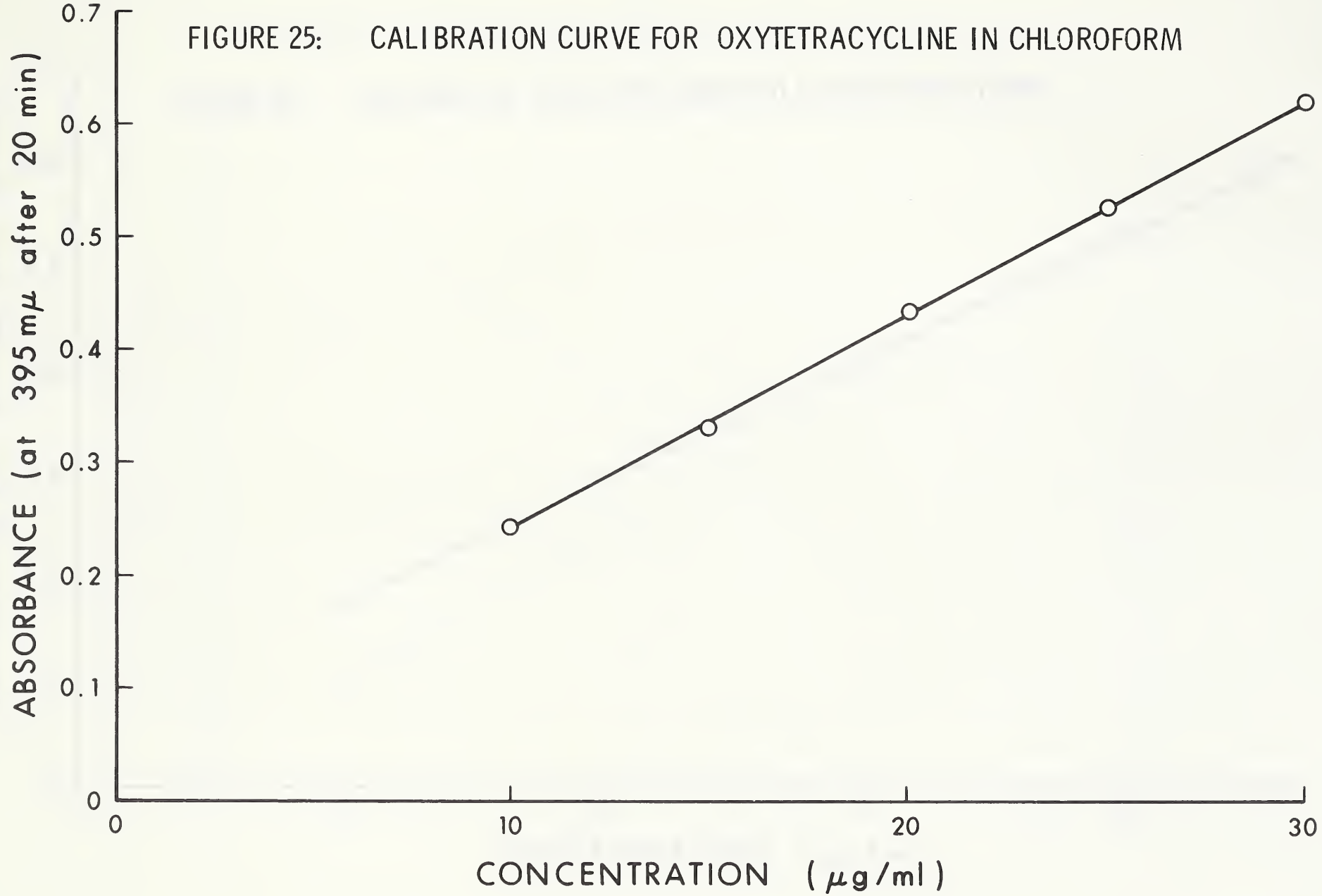


FIGURE 26: CALIBRATION CURVE FOR DEMETHYLCHLORTETRACYCLINE.

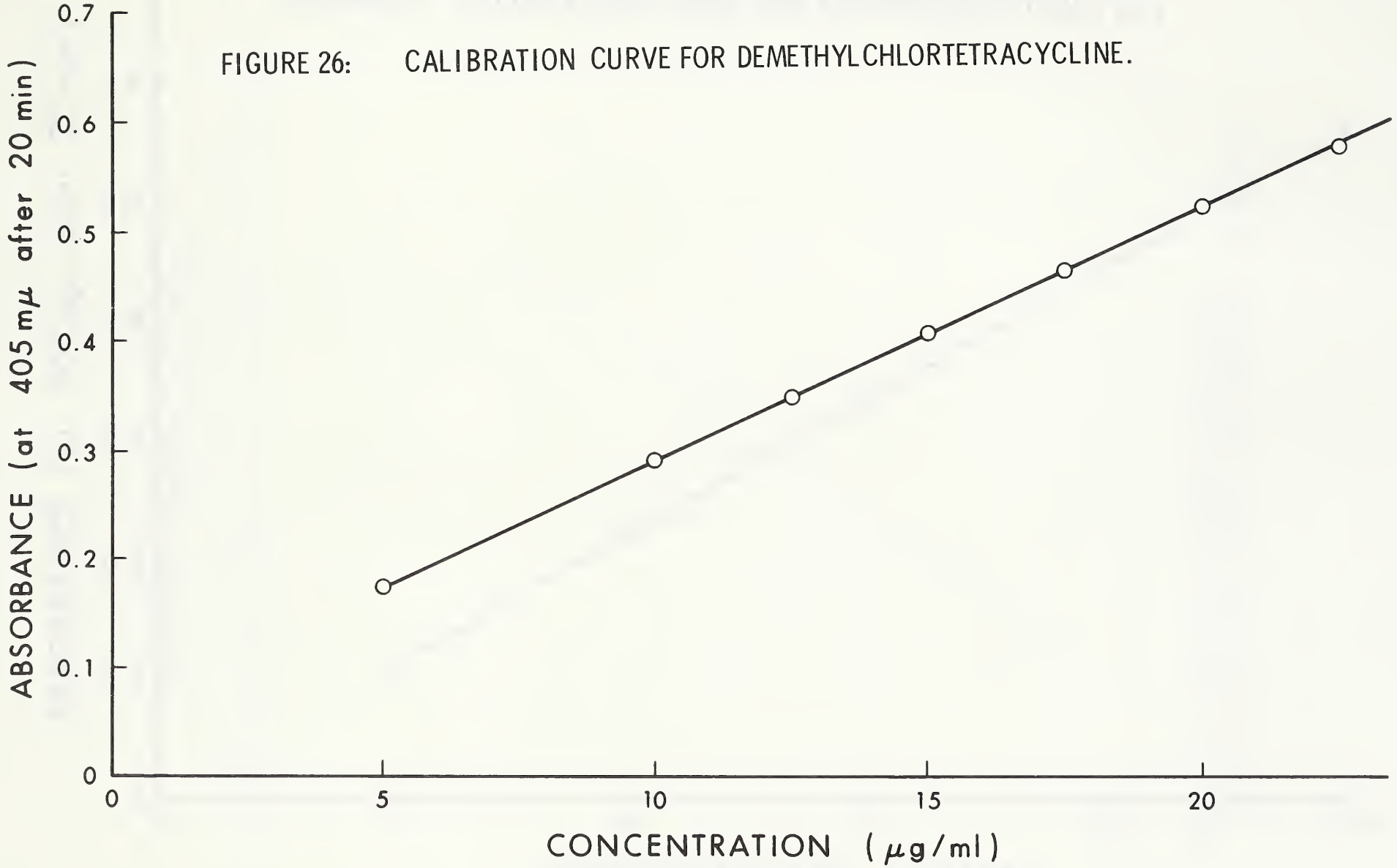


FIGURE 27: CALIBRATION CURVE FOR CHLORTETRACYCLINE HCl.

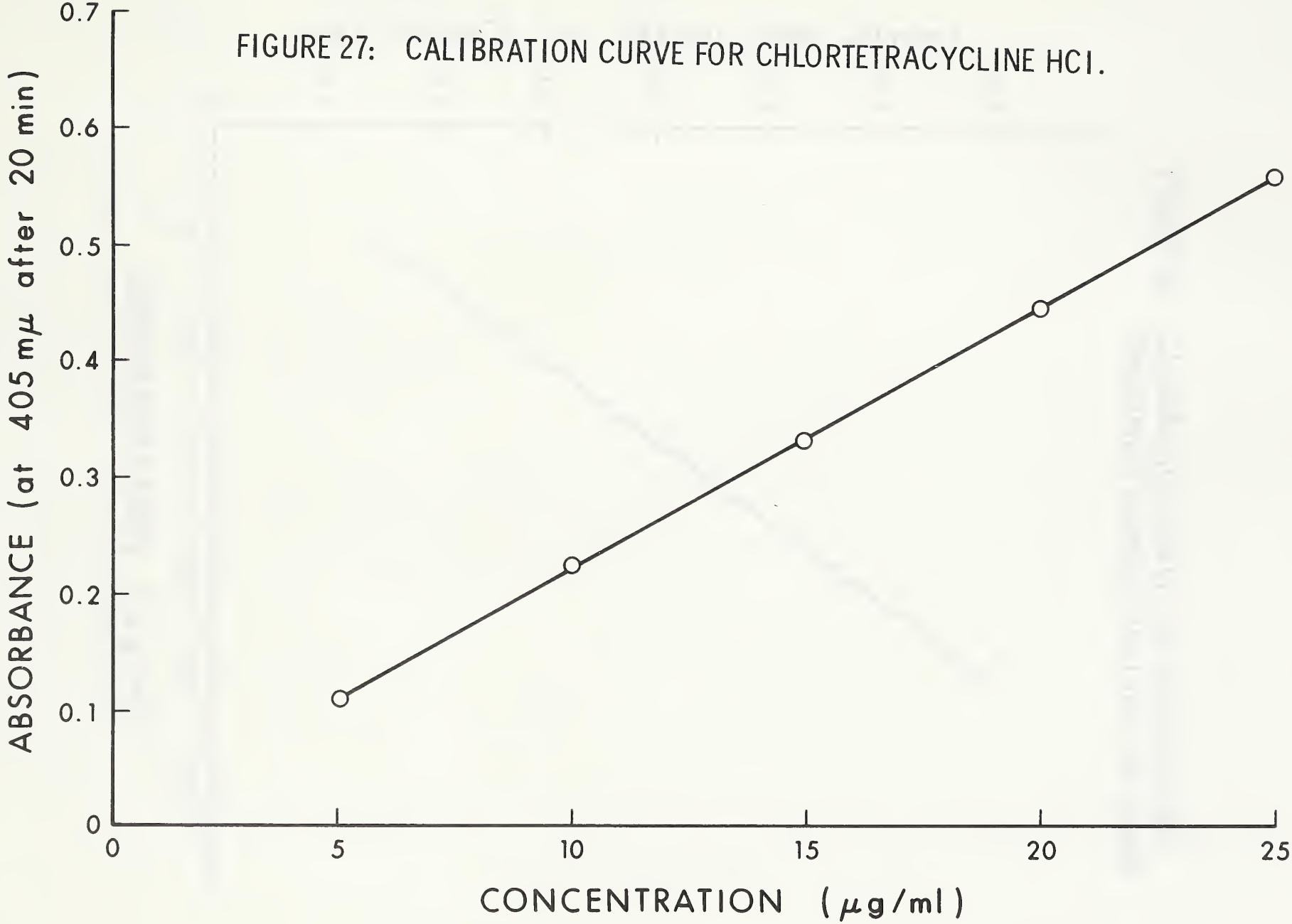
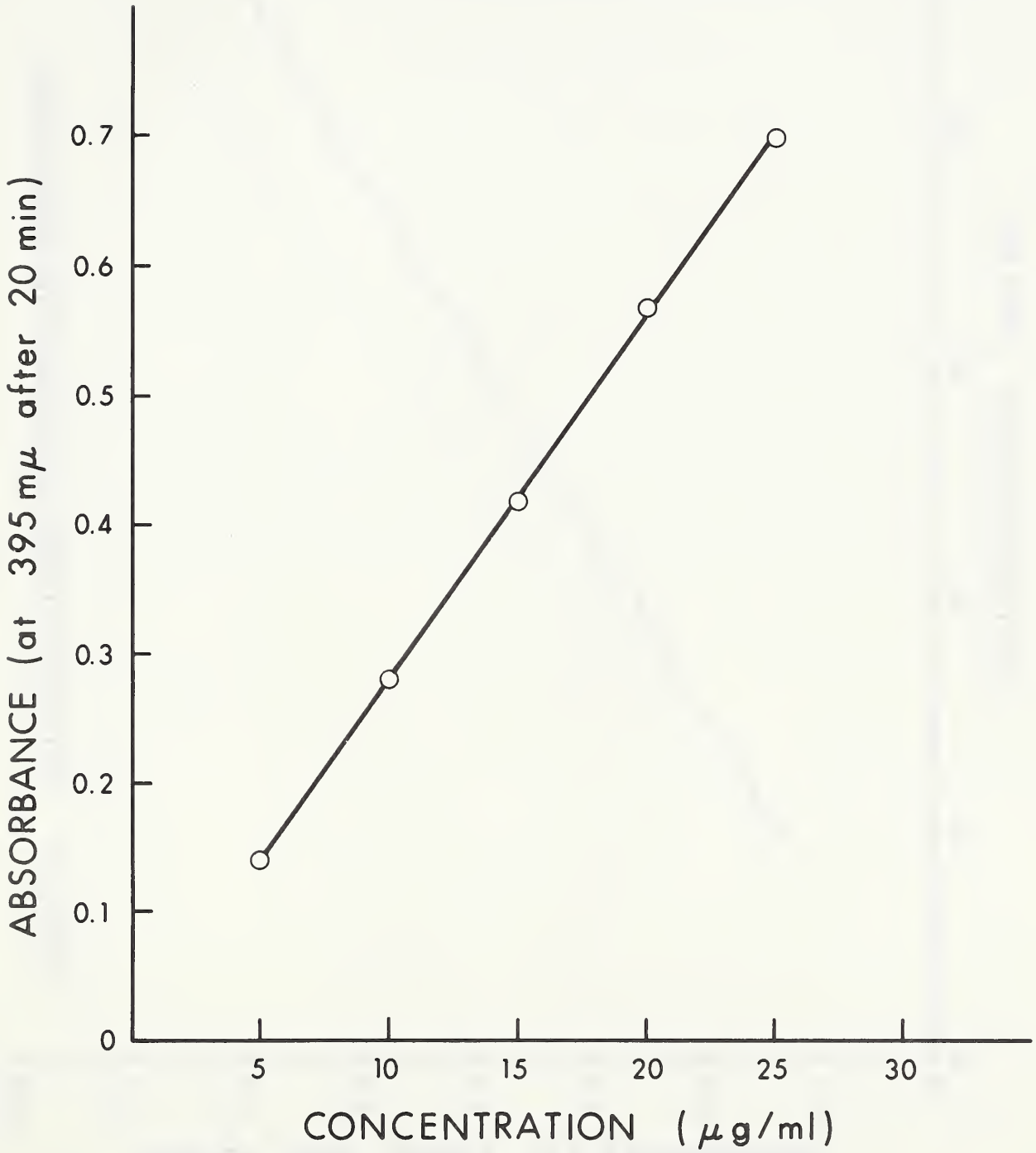


FIGURE 28: CALIBRATION CURVE FOR TETRACYCLINE PHOSPHATE COMPLEX (BRISTOL LOT C8688)



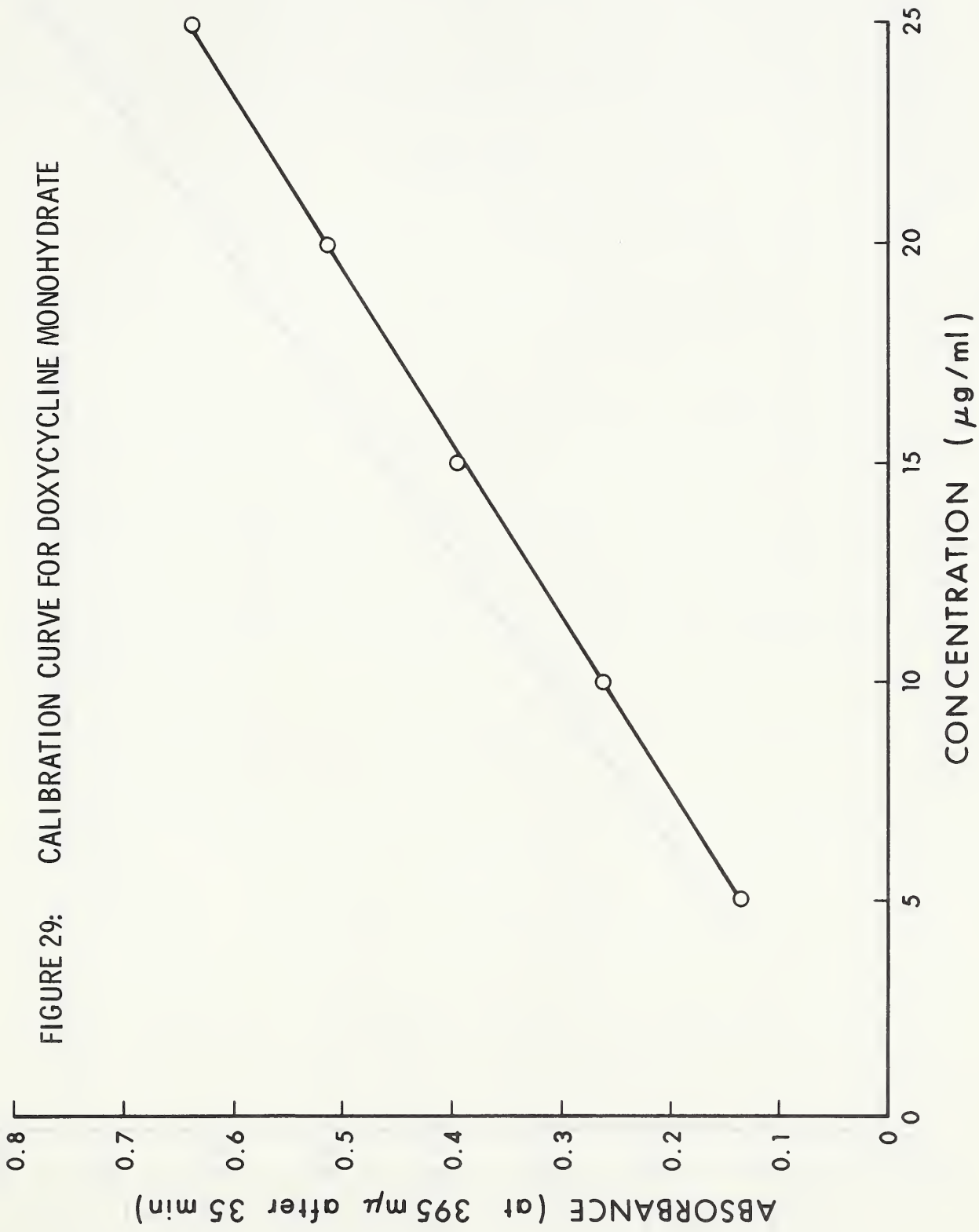


FIGURE 29: CALIBRATION CURVE FOR DOXYCYCLINE MONOHYDRATE

FIGURE 30: CALIBRATION CURVE FOR DOXYCYCLINE HCl.

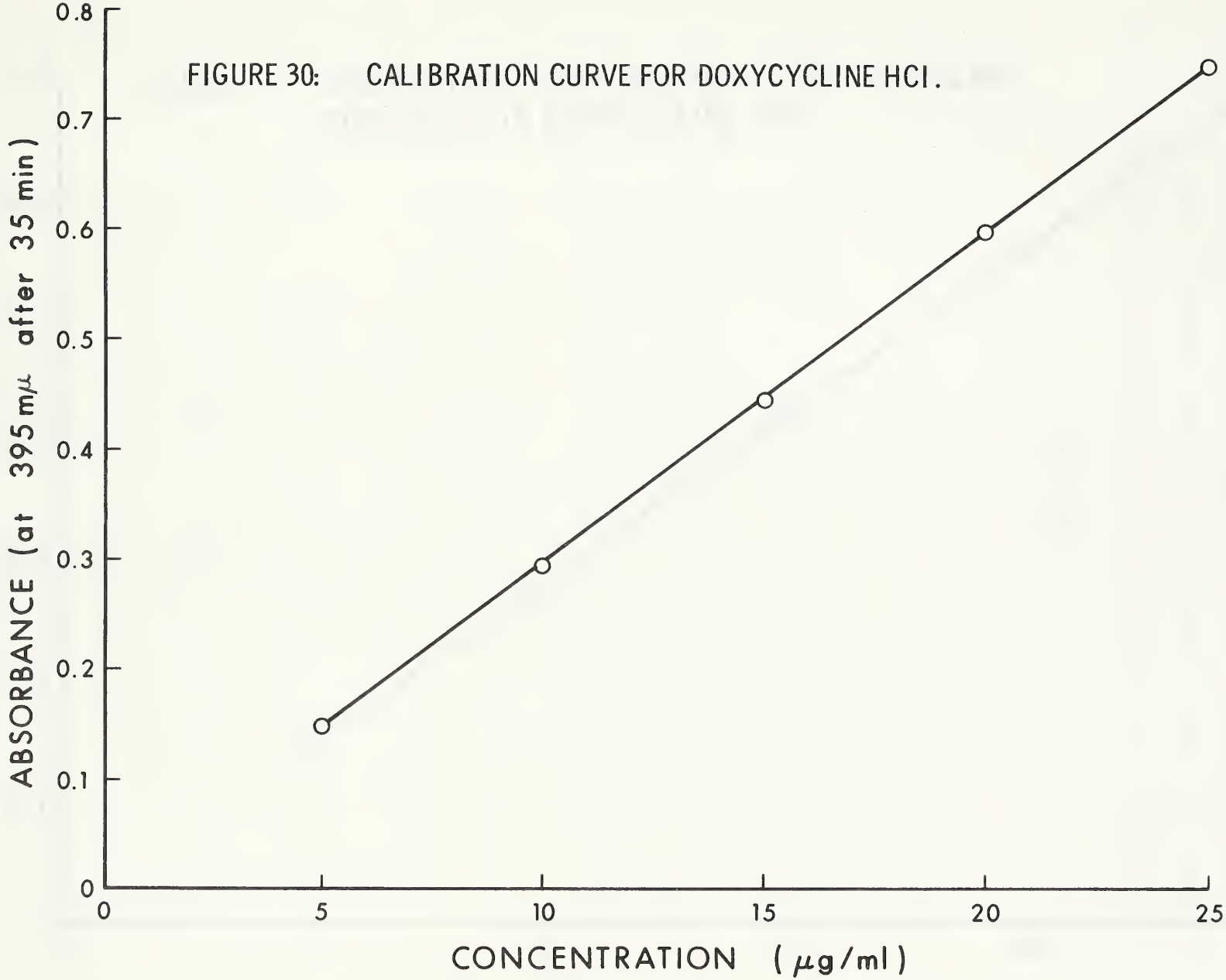
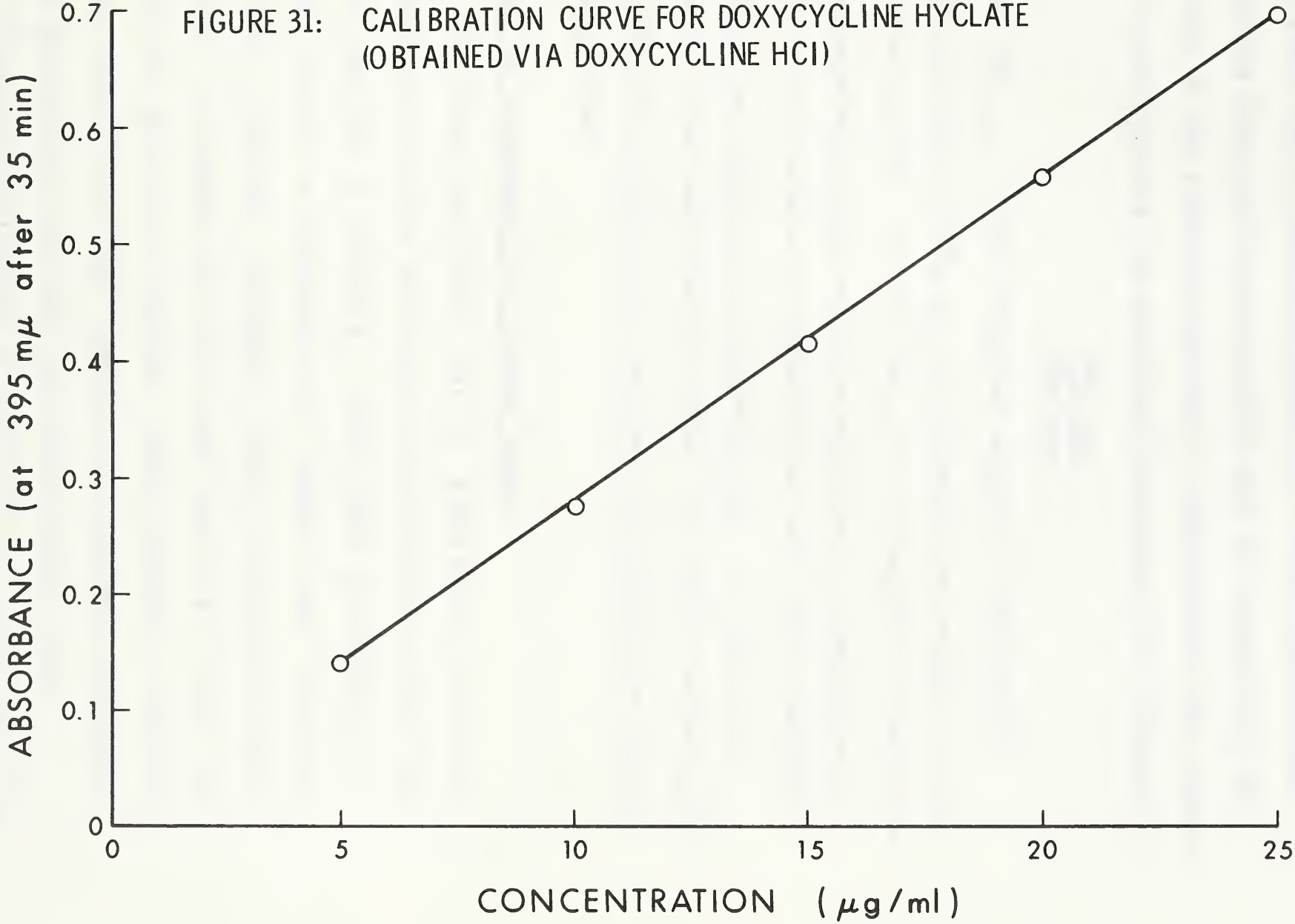


FIGURE 31: CALIBRATION CURVE FOR DOXYCYCLINE HYCLATE (OBTAINED VIA DOXYCYCLINE HCl)



its pure form. After correction of initial absorbances obtained using the hydrochloride salt to account for the purity of the reference material, extrapolation was achieved by multiplying the resultant absorbances by a factor of

$$\frac{MW_{D.HCl}}{MW_{D.HYC.}}$$

where $MW_{D.HCl}$ is the molecular weight of doxycycline hydrochloride and $MW_{D.HYC}$ is the molecular weight of doxycycline hyclate. In this way, it is possible to determine the absorbance which would be exhibited if doxycline hyclate at the same w/v concentration were being complexed rather than doxycycline hydrochloride. A linear calibration curve was obtained (Figure 31) which yielded quite satisfactory results for the assay of doxycycline hyclate dosage forms.

Assay of Pharmaceutical Dosage Forms

As shown previously (Part F, Experimental section), numerous procedures were derived by modification of two basic methods of analysis. Using these procedures, a large variety of pharmaceutical dosage forms including tablets, capsules, ointments, sprays, ophthalmic drops, syrups, and suspensions have been assayed for their tetracycline antibiotic content. Where possible, results of these assays have been compared with results obtained by the manufacturer for the individual dosage forms.

From an examination of Table IV, it can be seen that results obtained by the thorium complexation method agree

very favorably with the manufacturers' results for all tablets and capsules investigated. However, a few discrepancies appear in the comparison of results for some syrups and for Resteclin Aqueous Drops®. This can be generally attributed to a small degree of chemical alteration and degradation of the constituents of the preparation between the time of the manufacturer's assay and the thorium-complexation assay. The rate of chemical alteration of the tetracyclines is much more rapid when they are present in a liquid formulation, particularly in an aqueous medium, than when they are produced in dry, solid dosage forms such as tablets and capsules. Despite the fact that none of the liquid preparations had, at the time of this investigation, reached their designated expiry dates, the difference between the manufacturer's result (often in large excess of 100% labelled claim) and the result obtained by the present method was extremely large in some cases. These observations would indicate that the thorium complexation method may still represent a very sensitive assay procedure since the concentration of antibiotic originally placed in a liquid preparation is probably calculated such that the full labelled claim will be present at the expiry date.

Attempts to achieve good agreement with the manufacturer's result were unsuccessful for Terra-Cortril Spray®. Inaccuracies in the assay of this product probably resulted from the extreme difficulty in expelling the

Table IV. Results of Thorium Nitrate Colorimetric Method

Commercial Product	Manufacturer	Labelled Drug Content	% Labelled Strength	
			Colorimetric	Manufacturer's Result +
Achromycin Capsules ®	Lederle	231.04 mg tetracycline base per capsule	99.6	99.84
Albamycin T Capsules ®	Upjohn	115.52 mg tetracycline base per capsule	99.9	100.00
Aureomycin Capsules ®	Lederle	chlortetracycline HCl 250 mg/capsule	101.1	not available
Declostatin Capsules ®	Lederle	demethylchlortetracycline HCl 250 mg/capsule	97.7	99.90
Resteclin Capsules ®	Squibb	250 mg tetracycline base per capsule	101.5	118.00 (a) 101.20 (b)
Signemycin Capsules ®	Roerig	154.30 mg tetracycline base/capsule	99.5	108.90 (b)
Vibramycin Scripak Capsules ®	Pfizer	100 mg doxycycline base per capsule	100.7	not available

(a) microbiological assay result
(b) chemical assay result
+ Data supplied by manufacturer

Table IV cont'd...

Commercial Product	Manufacturer	Labelled Drug Content	% Labelled Strength	
			Colorimetric	Manufacturer's Result +
Achrocidin Tablets ®	Lederle	tetracycline HCl 125 mg/tablet	100.1	102.91
Aureomycin Soluble ® Tablets	Lederle	chlortetracycline HCl 50 mg/tablet	100.2	not available
Declomycin 300 Tablets ®	Lederle	demethylchlortetracycline HCl 300 mg/tablet	96.3	100.79
Achrocidin Cpd. Syrup ®	Lederle	tetracycline base 23.10 mg/cc	99.7	111.72
Declomycin Syrup ®	Lederle	demethylchlortetracycline base 73.60 mg/5 cc	98.4	121.83
Resteclin Syrup ®	Squibb	tetracycline base 23.10 mg/cc	99.4	116.80 (a) 136.80 (b)
Signemycin Syrup ®	Roerig	tetracycline base 15.43 mg/cc	101.2	119.70 (b)

(a) microbiological assay
(b) chemical assay method of manufacturer

+ Data supplied by manufacturer

Table IV cont'd...

Commercial Product	Manufacturer	Labelled Drug Content	% Labelled Strength: Colorimetric	% Labelled Strength: Manufacturer's Result +
Aureomycin Ear Solution ®	Lederle	chlortetracycline HCl 50 mg/vial	99.7	not available
Terra-Cortril Eye/Ear-Suspension ®	Pfizer	oxytetracycline base 5 mg/cc	98.9	100.00 (b)
Resteclin Aqueous Drops ®	Squibb	tetracycline base 92.40 mg/cc	100.0	109.10 (a)
Terra-Cortril Ointment ®	Pfizer	oxytetracycline base 30 mg/gram	91.6	not available
Aureomycin Ophthalmic Ointment ®	Lederle	chlortetracycline HCl 10 mg/gram	89.6	not available
Vibramycin Oral Suspension ®	Pfizer	doxycycline base 5 mg/cc when reconstituted	105.7	not available
Aureomycin Surgical Powder ®	Lederle	chlortetracycline HCl 200 mg/gram	99.6	not available

(a) microbiological assay
(b) chemical assay method of manufacturer

+ Data supplied by manufacturer

Table IV cont'd...

Commercial Product	Manufacturer	Labelled Drug Content	% Labelled Strength	
			Colorimetric	Manufacturer's Result +
Terra-Cortril Spray ®	Pfizer	oxytetracycline base 60 mg/cc (300 mg/can)	88.2	106.33 (b)
Tetrex Capsules ®	Bristol	tetracycline phosphate complex =250 mg tetracycline HCl/capsule	107.1	104.40 (b)
Tetrex-bidCaps ®	Bristol	tetracycline phosphate complex =500 mg tetracycline HCl/capsule	106.7	104.00 (b)
Tetrex-APC Capsules ®	Bristol	tetracycline phosphate complex =125 mg tetracycline HCl/capsule	110.8	110.40 (b)
Azotrex Capsules ®	Bristol	tetracycline phosphate complex =125 mg tetracycline HCl/capsule	107.7	112.00 (b)
Azotrex Syrup ®	Bristol	tetracycline phosphate complex =125 mg tetracycline HCl/5 cc	96.8	108.00 (b)

(a) microbiological assay
(b) chemical assay method of manufacturer

+ Data supplied by manufacturer

Table IV cont'd...

Commercial Product	Manufacturer	Labelled Drug Content	% Labelled Strength: Colorimetric	% Labelled Strength: Manufacturer's Result +
Tetrex Syrup ®	Bristol	tetracycline phosphate complex =125 mg tetracycline HCl/5 cc	103.9	100.00 (b)
Tetrex-AP Syrup ®	Bristol	tetracycline phosphate complex =125 mg tetracycline HCl/5 cc	99.3	104.00 (b)

(a) microbiological assay
(b) chemical assay method of manufacturer
+ Data supplied by manufacturer

entire contents of the spray can. Since the method employed (Experimental, part j) was based on the use of the total can contents, any residual liquid remaining in the container would necessarily yield low results.

For most of the pharmaceutical products, the results obtained by the thorium-complexation method were in satisfactory agreement with available results by comparative procedures as supplied by the manufacturers. The technique would seem to have a relatively high degree of applicability, being useful in the assay of a large variety of dosage forms, without interference from excipient material and secondary ingredients.

NMR Spectroscopy in the Analysis of Tetracyclines

It has been the purpose of this investigation to develop a new technique for the rapid and sensitive determination of tetracyclines in pharmaceutical dosage forms. In pursuing that objective, it is also within the scope of the present study to suggest an analytical technique which could lead to another accurate means of tetracycline estimation. The technique now being suggested is the use of nuclear magnetic resonance spectroscopy for quantitative analysis.

In recent years, the applications of NMR spectroscopy have been expanded beyond the area of structure elucidation to include numerous quantitative analytical procedures. From a pharmaceutical standpoint, NMR methods of analysis have already been employed for a number of products, in-

cluding APC mixtures, meprobamate tablets, and dimethylsulfoxide in solution and ointments.

The basic principle which permits the use of nuclear magnetic resonance absorption as a quantitative measure of a particular substance is that, in addition to factors such as relaxation time, intensity of applied magnetic field, and the field sweep rate, the signal strength is proportional to the number of protons giving rise to it. Therefore, if the intensity of an isolated peak of known origin in the NMR spectrum of a certain drug can be compared with one from a known quantity of an internal standard, quantitative estimation of the drug is made possible.

The spectrum of tetracycline hydrochloride in D_2O is given in Figure 32. It can be seen that in addition to the expected peaks in the aromatic region, three sharp singlets appear at 5.4τ, 6.97τ, and 8.45τ. The peak at 5.4τ can be readily attributed to the presence of water in the sample. Assignments of the remaining peaks are easily made by considering their integral values and field positions. The sharp peak at 6.97τ integrates for six protons and arises from the dimethylamino hydrogens in the 4-position substituent. At a higher field position and integrating for three protons, the singlet at 8.45τ originates from the three methyl protons contained in the six position methyl substituent. In the choice of a suitable analytical peak, the sharpness of the signal, preferably a singlet, and the subsequent ease of integral evaluation

are the major factors to be considered. Thus, in the spectrum of tetracycline, either the peak at 6.97 τ or the peak at 8.45 τ could feasibly be utilized for analytical purposes.

In all analytical NMR work, an internal standard must be employed in order to arrive at an accurate integral calibration. A known concentration of any proton-containing compound may be used as this standard, as long as a ratio of integral height to the number of protons can be definitely established. A suitable internal standard is one which gives rise to sharp signals of known origin which arise in a region of the spectrum such that no interference with other signals occurs.

Preliminary investigations in this study have indicated that maleic acid may be suitable as an internal standard in the quantitative analysis of tetracyclines. The spectrum of maleic acid in D_2O is shown in Figure 33, wherein it can be seen that the two equivalent methine protons of the acid give rise to a very sharp singlet at a low field position of 3.65 τ. By preparing a mixture of tetracycline hydrochloride and maleic acid and by examining the resultant NMR spectrum in D_2O (Figure 34), it becomes apparent that quantitative estimation of the tetracycline concentration in the prepared mixture is a relatively simple matter of mathematical calculation after the integral calibration has been established.

Two of the most apparent advantages in using NMR

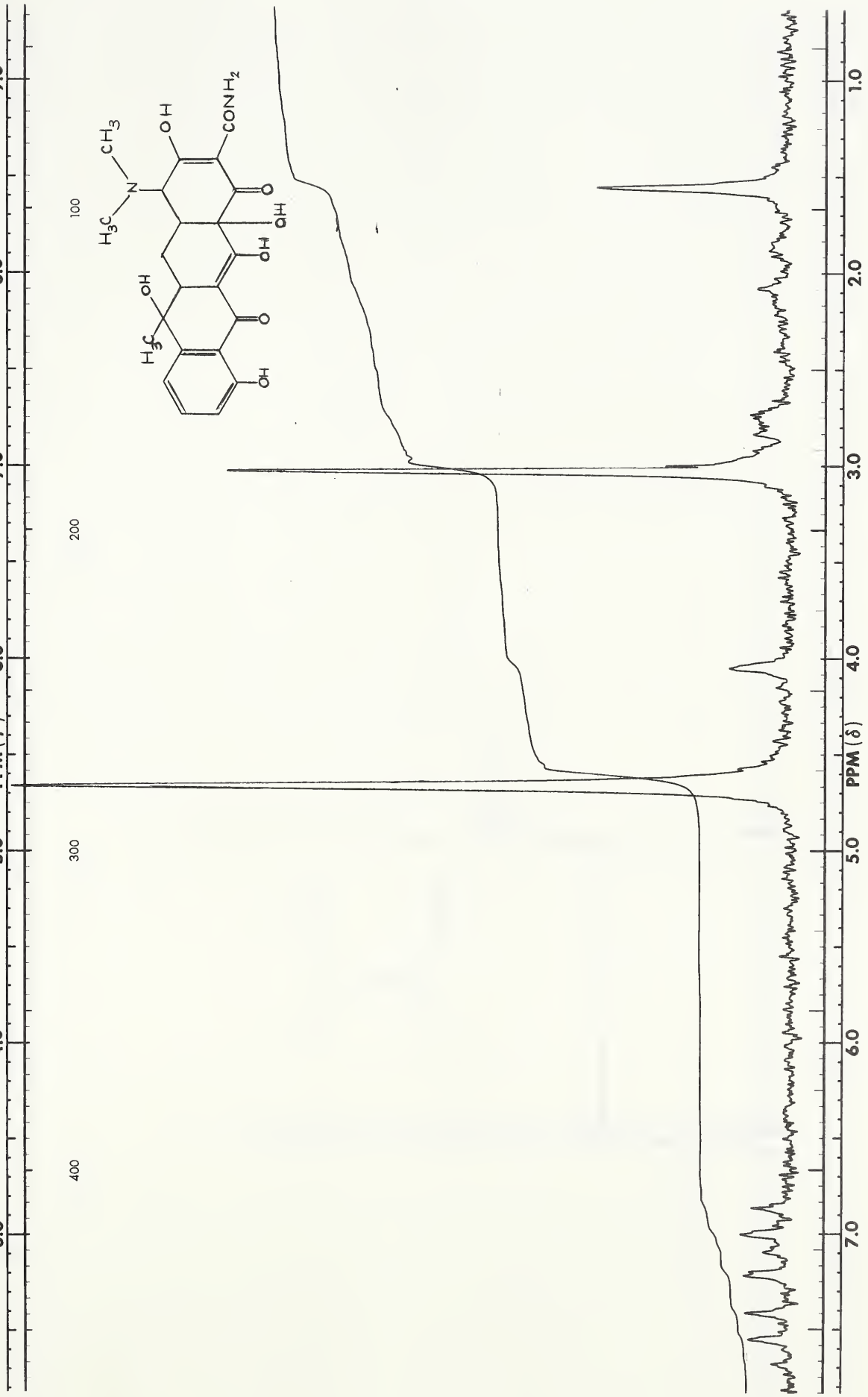


Figure 32. NMR Spectrum of Tetracycline HCl in D_2O

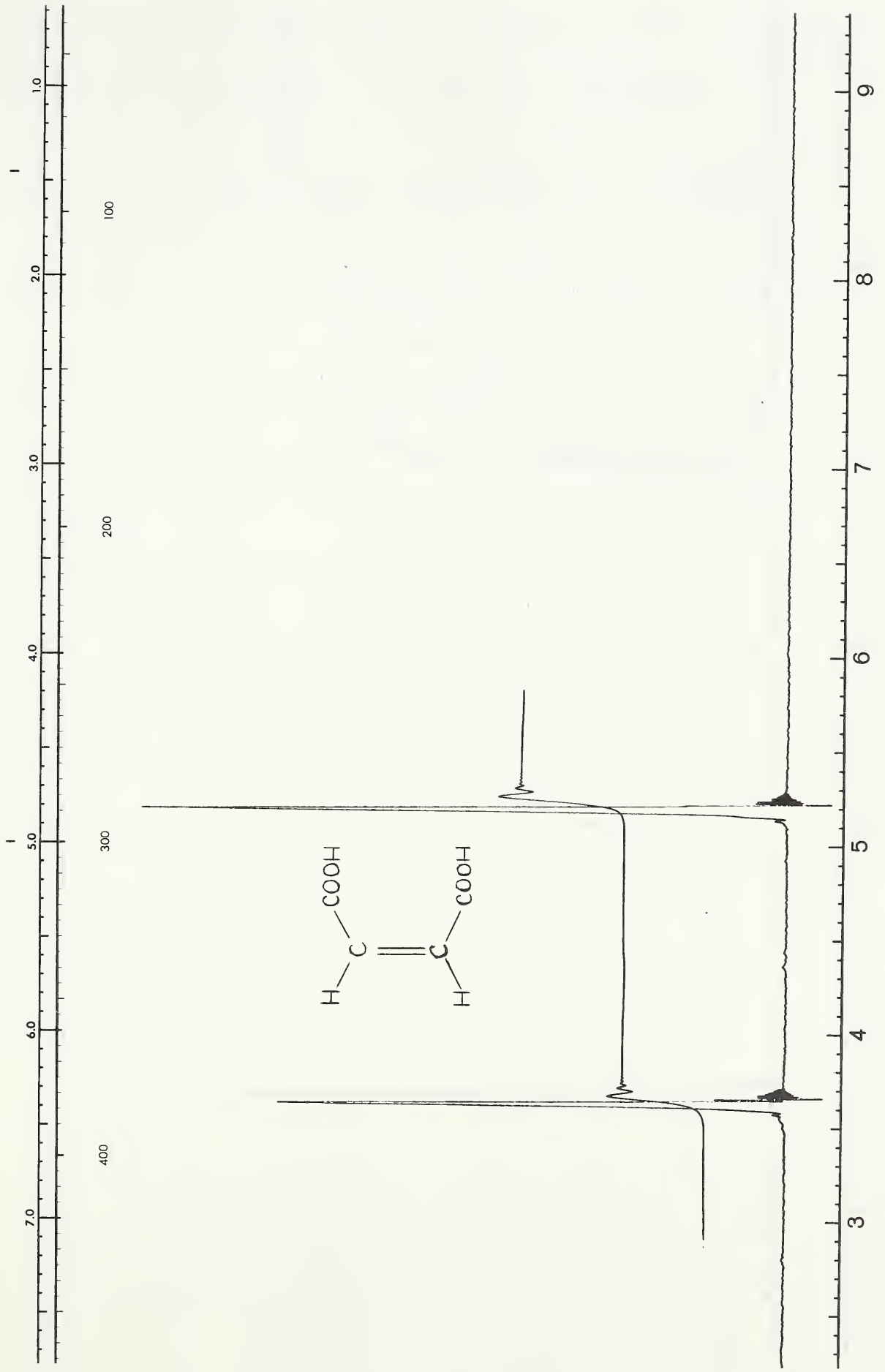


Figure 33. NMR Spectrum of Maleic Acid in D_2O

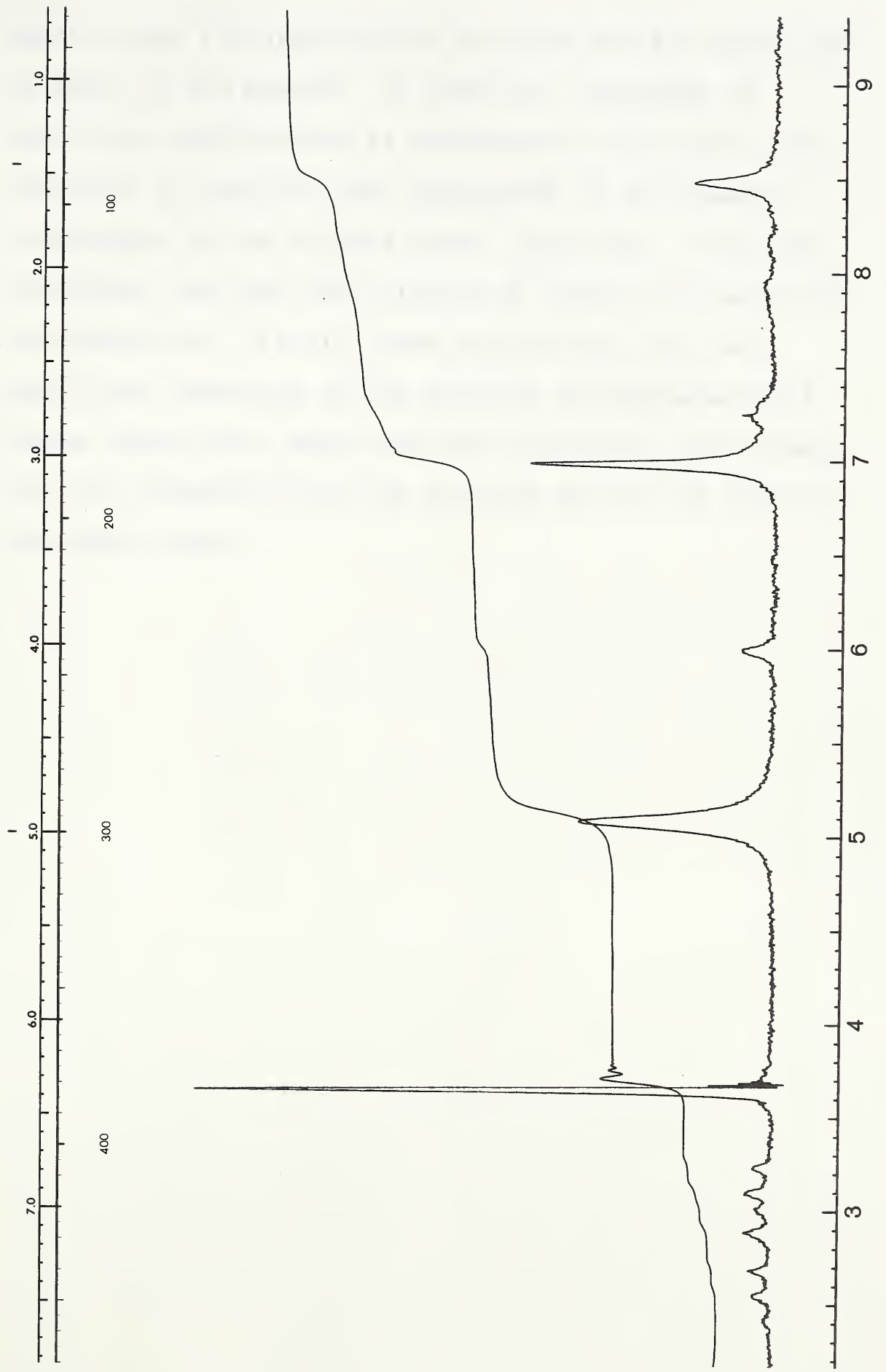


Figure 34. NMR Spectrum of Mixture of Tetracycline HCl (800 mg) and Maleic Acid (400 mg) in D_2O (5 ml)

spectroscopy for quantitative analysis are the speed and accuracy of the method. In addition, knowledge of extinction coefficients is unnecessary since such a coefficient is constant and independent of the chemical environment of the nucleus under inspection. This fact eliminates the need for calibration curves in the analytical procedure. Finally, NMR spectroscopy may be of particular advantage in the analysis of pharmaceutical dosage forms since many insoluble excipients and binders will not interfere with the analysis and can be left in the sample tube.

SUMMARY AND CONCLUSIONS

(1) A sensitive colorimetric method of analysis for 7 compounds of the tetracycline series and their pharmaceutical dosage forms has been developed which is based on the formation of a colored complex between the tetracyclines and thorium. The technique was applied to 29 pharmaceutical dosage forms and satisfactory results were obtained for most of these.

(2) The structure of the complex formed between tetracycline and thorium has been postulated and the suggested 2:2 stoichiometry of the complex has been confirmed by the molar ratio method.

(3) Studies of the effects of pH and time upon the stability of the colored complexes were conducted. From these studies it was found that a pH of 6.6 represented a good working value for all of the tetracyclines investigated and that a period of 20 to 35 minutes, depending on the particular compound, was required for stable color formation prior to spectrophotometric assay.

(4) Linear and reproducible calibration curves for 7 tetracyclines were obtained using pure reference materials supplied by the manufacturers. A theoretical calibration curve was also prepared for doxycycline hyclate by extrapolation of data pertaining to doxycycline HCl.

(5) It is concluded that the accuracy and reproducibility of the colorimetric method developed makes it superior to many procedures presently being employed for the analysis of tetracyclines.

(6) The possibility of applying nuclear magnetic resonance spectroscopy to the quantitative analysis of the tetracycline antibiotics has been illustrated. Maleic acid has been suggested as a suitable internal standard for future experimental work on this topic.

BIBLIOGRAPHY

(1) Goodman, L.S., and Gilman, A., The Pharmacological Basis of Therapeutics, Third Ed., The Macmillan Company, New York, N.Y., 1965, P.1242.

(2) Bourchier, W.F., Bristol Laboratories, Personal Communications, Sept., 1968.

(3) Kaplan, M.A., and Buckwalter, F.H., Antibiotics and Chemotherapy, 8, 361 (1968).

(4) Drug Merchandising Annual Survey of Prescriptions, Drug Merchandising, 15 (July, 1969).

(5) The British Pharmacopoeia, The Pharmaceutical Press, 17 Bloomsbury Square, London, England, 1968.

(6) Garrett, E.R., J. Pharm. Sci., 51, 1036 (1962).

(7) Selzer, G.B., and Wright, W.W., Antibiot. Chemotherapy, 7, 292 (1957).

(8) Kelly, R.G., and Buyske, D.A., ibid, 10, 604 (1960).

(9) Addison, E., and Clark, R.G., J. Pharm. and Pharmacol., 15, 268 (1963).

(10) Ascione, P.P., Zagar, J.B., and Chrekian, G.P., J. Pharm. Sci., 56, 1396 (1967).

(11) Sideri, C.N., and Osol, A., J. Am. Pharm. Assoc., Sci. Ed., 42, 688 (1953).

(12) Yokoyama, F., and Chatten, L.G., J. Am. Pharm. Assoc., Sci. Ed., 47, 548 (1958).

(13) Kohn, K.W., Anal. Chem., 33, 862 (1961).

(14) Chiccarelli, F.S., and Woolford, M.H., Jr., J. Am. Pharm. Assoc., Sci. Ed., 45, 400 (1956).

(15) Chiccarelli, F.S., Woolford, M.H., Jr., and Avery, M.E., J. Am. Pharm. Assoc., Sci. Ed., 48, 263 (1959).

(16) Monastero, F., Means, J.A., Grenfell, T.C., and Hedgerr, F.H., J. Am. Pharm. Assoc., Sci. Ed., 40, 241 (1951).

(17) Grenfell, T.C., McLaughlin, D.I., and Lacques, M. Kelly, Ann. New York Acad. Sci., 87, 857 (1960).

(18) Mazo, L., and Papay, M., Z. anal. Chem., 175, 355 (1960).

(19) Kakemi, K., Uno, T., and Samejima, M., J. Pharm. Soc. Japan, 75, 970 (1955).

(20) Uno, T., and Morishita, N., J. Pharm. Soc., Japan, 80, 1679 (1960).

(21) Kakemi, K., Uno, T., and Miyake, N., J. Pharm. Soc. Japan, 75, 973 (1955).

(22) Pligin, S.G., Zh. analit. Khim., 22, 1008 (Russian), 925 (English) (1967).

(23) Sakaguchi, T., and Taguchi, K., Pharm. Bull., Japan, 3, 166 (1955).

(24) Sakaguchi, T., Taguchi, K., and Suzuki, A., Japan Analyst, 6, 782 (1957), through Anal. Abst., 5, 3502 (1958).

(25) Ryabchikov, D.I., and Gol'braikh, E.K., The Analytical Chemistry of Thorium, International Series of Monographs on Analytical Chemistry, 10, 4 (1963).

(26) Moeller, T., Inorganic Chemistry, John Wiley and Sons, Inc., New York, N.Y., 1958, P.104.

(27) Ryabchikov, D.I., and Gol'braikh, E.K., The Analytical Chemistry of Thorium, International Series of Monographs on Analytical Chemistry, 10, 71 (1963).

APPENDIX

Table V. Data for Analysis of Tetracycline Reference Standards by Nonaqueous Titration

Drug	Sample wt. (mg)	Volume Titrant (ml)	mg recovered	% recovered	Average Purity (%)
Tetracycline HCl (a)	145.67	6.10	141.65	97.24	
	193.07	8.10	188.07	97.41	97.32
	183.83	7.70	178.89	97.31	
Oxytetracycline HCl (a)	152.11	6.30	151.23	99.42	
	205.00	8.50	204.02	99.52	99.50
	183.21	7.60	182.40	99.56	
Chlortetracycline HCl (a)	130.30	5.00	124.47	95.52	
	113.20	4.40	109.53	96.76	96.12
	139.90	5.40	134.42	96.08	
Demethylchlortetracycline HCl (a)	134.40	5.40	130.75	97.28	
	146.30	5.90	142.83	97.63	97.76
	157.50	6.40	154.96	98.38	
Tetracycline phophate complex	-	-	-	-	-
Doxycycline HCl (b)	246.10	9.40	232.32	94.40	
	180.20	6.95	171.72	95.30	94.80
	199.30	7.65	189.03	94.80	

(a) Titrant used = 0.0483 N $HClO_4$

(b) Titrant used = 0.0514 N $HClO_4$

Table V cont'd...

Drug	Sample wt. (mg)	Volume Titrant (ml)	mg recovered	% recovered	Average Purity (%)
Doxycycline mono-hydrate (b)	200.20	8.50	202.07	100.91	
	126.02	5.30	125.96	99.95	100.29
	136.61	5.75	136.64	100.02	

(a) Titrant used = 0.0483 N $HClO_4$

(b) Titrant used = 0.0514 N $HClO_4$

Table VI. Data for Molar Ratio Study of Tetracycline-Thorium System[a]

mls 0.001 M $Th(NO_3)_4$ added	Mole Fraction of $Th(NO_3)_4$	Absorbance at 395 mμ
0.5	0.125	0.105
1.0	0.250	0.200
1.5	0.375	0.270
2.0	0.500	0.320
2.5	0.625	0.325
3.0	0.750	0.325

(a) Concentration of tetracycline hydrochloride held constant at 2.0×10^{-6} moles per 100 ml.

Table VII. Data for Determination of Wavelength of Maximum Absorbance

Compound		O.D. at 375 mμ	O.D. at 380 mμ	O.D. at 385 mμ	O.D. at 390 mμ	O.D. at 395 mμ	O.D. at 400 mμ	O.D. at 405 mμ
Tetracycline HCl	#1	0.240	0.395	0.510	0.600	0.630	0.630	0.590
	#2	0.200	0.390	0.510	0.590	0.630	0.625	0.585
Chlortetracycline HCl	#1	0.115	0.205	0.285	0.365	0.420	0.455	0.470
	#2	0.120	0.210	0.290	0.370	0.425	0.460	0.475
Oxytetracycline HCl	#1	0.280	0.385	0.460	0.500	0.510	0.480	0.400
	#2	0.285	0.390	0.465	0.505	0.515	0.485	0.400
Demethylchlor-tetracycline HCl	#1	0.060	0.175	0.285	0.390	0.450	0.500	0.510
	#2	0.060	0.170	0.285	0.385	0.440	0.500	0.505
Tetracycline PO_4 complex	#1	0.220	0.320	0.390	0.430	0.445	0.425	0.360
	#2	0.220	0.315	0.390	0.425	0.430	0.395	0.330
Doxycycline HCl	#1	0.300	0.360	0.400	0.420	0.425	0.390	0.330
	#2	0.290	0.350	0.400	0.425	0.430	0.395	0.330
Doxycycline monohydrate	#1	0.290	0.365	0.410	0.440	0.445	0.410	0.350
	#2	0.285	0.365	0.415	0.450	0.455	0.420	0.365

Table VII cont'd....

Compound		O.D. at 410 mμ	O.D. at 415 mμ	O.D. at 420 mμ	O.D. at 425 mμ	O.D. at 430 mμ	O.D. at 435 mμ
Tetracycline HCl	#1	0.590	0.345	0.240	0.115	0.065	0.035
	#2	0.590	0.340	0.235	0.115	0.065	0.030
Chlortetracycline HCl	#1	0.445	0.385	0.315	0.230	0.160	0.110
	#2	0.445	0.385	0.315	0.230	0.160	0.110
Oxytetracycline HCl	#1	0.300	0.200	0.125	0.070	0.040	0.025
	#2	0.300	0.200	0.125	0.060	0.035	0.020
Demethylchlor-tetracycline HCl	#1	0.480	0.410	0.300	0.200	0.120	0.065
	#2	0.480	0.410	0.300	0.200	0.115	0.060
Tetracycline PO_4 complex	#1	0.250	0.165	0.090	0.050	0.030	0.020
	#2	0.255	0.160	0.090	0.050	0.030	0.020
Doxycycline HCl	#1	0.250	0.170	0.110	0.065	0.035	0.015
	#2	0.250	0.165	0.110	0.070	0.040	0.020
Doxycycline monohydrate	#1	0.275	0.195	0.130	0.075	0.040	0.020
	#2	0.280	0.215	0.140	0.080	0.045	0.020

Table VIII. Effect of pH on Color Development

1. Tetracycline Hydrochloride O.D. at 395 mμ

pH	Time (min) 5	10	15	20	25	30	35	40	45
2.0	0.290	0.290	0.290	0.290	0.290	0.290	0.290	0.290	0.290
3.0	0.620	0.610	0.600	0.590	0.580	0.580	0.575	0.575	0.575
4.0	0.630	0.630	0.620	0.615	0.615	0.600	0.590	0.580	0.580
5.0	0.510	0.530	0.535	0.535	0.540	0.540	0.540	0.540	0.540
6.0	0.550	0.550	0.545	0.540	0.530	0.525	0.525	0.525	0.525
6.6 (distilled water)	0.600	0.600	0.610	0.620	0.620	0.620	0.620	0.620	0.620

Table VIII cont'd...

2. Oxytetracycline Hydrochloride O.D. at 395 mμ

pH	Time (min) 5	10	15	20	25	30	35	40	45
2.0	0.275	0.280	0.280	0.285	0.290	0.290	0.290	0.290	0.290
3.0	0.530	0.530	0.520	0.520	0.510	0.500	0.500	0.500	0.490
4.0	0.560	0.550	0.530	0.530	0.510	0.510	0.505	0.505	0.500
5.0	0.480	0.485	0.475	0.470	0.470	0.470	0.470	0.460	0.450
6.0	0.390	0.370	0.370	0.360	0.360	0.360	0.360	0.360	0.360
6.6 (distilled water)	0.430	0.440	0.440	0.445	0.445	0.445	0.445	0.445	0.445

Table VIII cont'd...

3. Chlortetracycline HCl O.D. at 405 mμ

pH	Time (min) 5	10	15	20	25	30	35	40	45	50
2.0	0.325	0.230	0.225	0.205	0.195	0.185	0.180	0.175	0.165	0.165
3.0	0.410	0.425	0.425	0.420	0.420	0.410	0.410	0.410	0.405	0.400
4.0	0.435	0.460	0.460	0.460	0.460	0.460	0.450	0.450	0.450	0.445
5.0	0.360	0.385	0.390	0.390	0.395	0.400	0.390	0.390	0.390	0.390
6.0	0.340	0.340	0.340	0.340	0.340	0.340	0.330	0.330	0.330	0.330
6.6 (distilled water)	0.310	0.320	0.350	0.370	0.370	0.370	0.370	0.370	0.370	0.365

Table VIII cont'd...

4. Demethylchlortetracycline Hydrochloride O.D. at 405 mμ

pH	Time (min) 5	10	15	20	25	30	35	40	45	50
2.0	0.305	0.295	0.290	0.290	0.290	0.290	0.290	0.290	0.290	0.285
3.0	0.280	0.275	0.275	0.275	0.270	0.270	0.265	0.265	0.260	0.260
4.0	0.280	0.270	0.265	0.265	0.255	0.255	0.250	0.250	0.240	0.240
5.0	0.220	0.205	0.200	0.195	0.190	0.185	0.185	0.185	0.185	0.185
6.0	0.360	0.310	0.300	0.295	0.290	0.285	0.285	0.285	0.285	0.285
6.6 (distilled water)	0.385	0.385	0.385	0.380	0.380	0.380	0.380	0.380	0.380	0.375

Table VIII cont'd...

5. Tetracycline Phosphate Complex O.D. at 395 mμ

pH	Time (min) 5	10	15	20	25	30	35	40	45	50
2.0	0.175	0.185	0.185	0.180	0.180	0.180	0.180	0.180	0.180	0.180
3.0	0.350	0.360	0.360	0.355	0.355	0.355	0.355	0.355	0.355	0.355
4.0	0.410	0.410	0.410	0.400	0.400	0.400	0.400	0.395	0.395	0.395
5.0	0.340	0.355	0.355	0.355	0.350	0.350	0.350	0.350	0.350	0.350
6.0	0.330	0.335	0.335	0.330	0.325	0.325	0.325	0.325	0.325	0.320
6.6 (distilled water)	0.370	0.370	0.380	0.380	0.380	0.380	0.380	0.380	0.380	0.380

Table VIII cont'd...

6. Doxycycline Monohydrate O.D. at 395 mμ

pH	Time (min) 5	10	15	20	25	30	35	40	45	50
2.0	0.370	0.375	0.380	0.375	0.375	0.370	0.370	0.370	0.370	0.370
3.0	0.280	0.280	0.280	0.275	0.275	0.270	0.270	0.270	0.270	0.270
4.0	0.305	0.305	0.300	0.300	0.310	0.300	0.300	0.300	0.300	0.300
5.0	0.335	0.330	0.320	0.320	0.310	0.310	0.310	0.310	0.310	0.310
6.0	0.390	0.375	0.375	0.375	0.380	0.380	0.380	0.380	0.380	0.380
6.6 (distilled water)	0.440	0.465	0.470	0.470	0.470	0.475	0.475	0.475	0.475	0.475

Table VIII cont'd...

7. Doxycycline Hydrochloride O.D. at 395 mμ

pH	Time (min) 5	10	15	20	25	30	35	40	45	50
2.0	0.260	0.270	0.270	0.270	0.270	0.270	0.270	0.270	0.265	0.265
3.0	0.580	0.570	0.565	0.560	0.560	0.560	0.560	0.560	0.560	0.560
4.0	0.610	0.610	0.610	0.610	0.600	0.600	0.600	0.600	0.600	0.600
5.0	0.480	0.485	0.490	0.490	0.500	0.500	0.500	0.500	0.490	0.490
6.0	0.520	0.540	0.550	0.550	0.550	0.550	0.550	0.550	0.550	0.550
6.6 (distilled water)	0.630	0.630	0.620	0.620	0.620	0.620	0.620	0.620	0.620	0.620

Table IX. Effect of Time on Color Development

Time (min)	Tetracycline HCl O.D at 395 mμ	Oxytetracycline HCl O.D.at 395 mμ	Chlortetracycline HCl O.D.at 405 mμ	Demethylchlortetracycline O.D.at 405 mμ	Tetracycline PO_4 complex O.D.at 395 mμ	Doxycycline HCl O.D.at 395 mμ	Doxycycline monohydrate O.D.at 395 mμ
5	0.590	0.700	0.570	0.480	0.425	0.410	0.435
10	0.590	0.720	0.620	0.485	0.425	0.420	0.440
15	0.595	0.730	0.630	0.485	0.425	0.420	0.445
20	0.595	0.730	0.630	0.485	0.430	0.420	0.450
25	0.595	0.730	0.630	0.485	0.430	0.425	0.450
30	0.595	0.730	0.630	0.485	0.430	0.430	0.500
35	0.595	0.730	0.630	0.485	0.430	0.430	0.500
40	0.595	0.730	0.630	0.485	0.430	0.430	0.500
45	0.595	0.725	0.630	0.485	0.430	0.430	0.500
50	0.595	0.725	0.630	0.475	0.430	0.430	0.500
55	0.595	0.725	0.625	0.470	0.430	0.430	0.500

Table IX cont'd....

Time (min)	Tetracycline HCl	Oxytetracycline HCl	Chlortetracycline HCl	Demethylchlortetracycline	Tetracycline PO_4 complex	Doxycycline HCl	Doxycycline mono hydrate
	O.D.at 395 mμ	O.D.at 395 mμ	O.D.at 405 mμ	O.D.at 405 mμ	O.D.at 395 mμ	O.D.at 395 mμ	O.D.at 395 mμ
60	0.590	0.720	0.620	0.470	0.425	0.430	0.500
65	0.590	0.720	0.615	0.470	0.425	0.430	0.500
70	0.590	0.720	0.615	0.470	0.425	0.430	0.500
75	0.590	0.720	0.615	0.470	0.425	0.430	0.500

Table X. Data for Calibration Curves

Compound	Conc. of Drug (μg/ml)	λmax	O.D. #1	O.D. #2	O.D. #3	O.D. Av	Correction Factor	Corrected Average O.D.
Tetracycline base	5	395 mμ	0.190	0.210	0.200	0.200	100/97.32	0.210
	10		0.400	0.400	0.400	0.400		0.400
	15		0.620	0.620	0.615	0.618		0.640
	20		0.800	0.810	0.820	0.810		0.830
	25		0.990	0.990	0.990	0.990		1.020
Oxytetracycline base (in aqueous solution)	10	395 mμ	0.334	0.336	0.335	0.335	100/99.58	0.336
	15		0.490	0.500	0.500	0.497		0.502
	20		0.640	0.660	0.680	0.660		0.662
	25		0.820	0.820	0.820	0.820		0.823
	30		0.960	0.960	0.960	0.960		0.964
Oxytetracycline base (in chloroform)	10	395 mμ	0.240	0.235	0.240	0.238	100/99.58	0.241
	15		0.330	0.330	0.330	0.330		0.331
	20		0.450	0.420	0.430	0.433		0.432
	25		0.520	0.520	0.515	0.518		0.522
	30		0.610	0.610	0.610	0.610		0.613
Chlortetracycline HCl	5	405 mμ	0.110	0.110	0.110	0.110	100/96.12	0.114
	10		0.220	0.225	0.220	0.222		0.230
	15		0.330	0.330	0.330	0.330		0.343
	20		0.440	0.440	0.440	0.440		0.458
	25		0.550	0.550	0.550	0.552		0.574

Table X cont'd...

Compound	Conc. of Drug (µg/ml)	λmax	O.D. #1	O.D. #2	O.D. #3	O.D. Av	Correction Factor	Corrected Average O.D.
Demethylchlor-tetracycline base	5	405 mµ	0.169	0.173	0.171	0.171	100.0/97.76	0.175
	10		0.284	0.284	0.285	0.284		0.290
	15		0.395	0.400	0.400	0.398		0.400
	20		0.500	0.505	0.510	0.505		0.520
	25		0.580	0.590	0.580	0.583		0.590
Tetracycline phosphate complex	5	395 mµ	0.140	0.135	0.140	0.138	-	-
	10		0.270	0.270	0.300	0.280		-
	15		0.420	0.420	0.420	0.420		-
	20		0.570	0.560	0.580	0.570		-
	25		0.700	0.700	0.700	0.700		-
Doxycycline monohydrate	5	395 mµ	0.135	0.130	0.135	0.133	100.0/100.29	0.130
	10		0.260	0.260	0.260	0.260		0.260
	15		0.405	0.400	0.380	0.395		0.390
	20		0.510	0.515	0.515	0.513		0.510
	25		0.640	0.630	0.640	0.633		0.630
Doxycycline HCl	5	395 mµ	0.142	0.140	0.141	0.141	100.0/94.80	0.149
	10		0.280	0.280	0.275	0.278		0.293
	15		0.415	0.420	0.420	0.418		0.441
	20		0.560	0.565	0.560	0.562		0.593
	25		0.710	0.700	0.705	0.705		0.744

Table X cont'd...

Compound	Conc. of Drug (µg/ml)	λmax	O.D. #1	O.D. #2	O.D. #3	O.D. Av	Correction Factor	Corrected Average O.D.
Doxycycline hyclate	5	395 mu				0.149	480.90/ 512.94	0.140
	10					0.293		0.275
	15					0.441		0.413
	20					0.593		0.556
	25					0.744		0.698

Table XI. Data for Analysis of Pharmaceutical Dosage Forms

(excluding tetracycline phosphate complex products)

Product	Labelled Drug Content	Theoretical conc. final dilution (µg/ml)	O.D.	Found conc. of final dilution (µg/ml)	% Labelled Strength	Mean
Albamycin T Capsules®	115.52 mg TC[a] base/capsule	13.86	0.570	13.80	99.6	
			0.570	13.80	99.6	
			0.575	13.90	100.3	99.9
			0.575	13.90	100.3	±0.4
			0.570	13.80	99.6	
Signemycin Syrup®	15.43 mg TC base/cc	13.86	0.580	14.00	101.0	101.2
			0.580	14.00	101.0	±0.5
			0.585	14.15	102.1	
			0.580	14.00	101.0	
			0.580	14.00	101.0	
Signemycin Capsules®	154.3 mg TC base/capsule	15.00	0.615	14.85	99.0	99.5
			0.620	15.00	100.0	±0.5
			0.620	15.00	100.0	
			0.615	14.85	99.0	
			0.615	14.85	99.0	
			0.620	15.00	100.0	
Achrocidin Cpd. Syrup®	23.1 mg TC base/ml	13.86	0.575	13.80	99.8	99.7
			0.570	13.70	99.6	±0.1
			0.570	13.70	99.6	
			0.570	13.70	99.6	
			0.575	13.80	99.8	

(a) Tetracycline is abbreviated as TC.

Table XI cont'd...

Product	Labelled Drug Content	Theoretical conc. final dilution (µg/ml)	O.D.	Found conc. of final dilution (µg/ml)	% Labelled Strength	Mean
Achrocidin Tablets®	115.52 mg TC[a] base/tablet	15.00	0.620	15.00	100.0	100.1
			0.620	15.00	100.0	±0.9
			0.615	14.85	99.0	
			0.620	15.00	100.0	
			0.630	15.25	101.7	
Resteclin Caps.®	250 mg TC base/capsule	15.00	0.630	15.30	102.0	101.5
			0.625	15.10	100.5	±0.8
			0.630	15.30	102.0	
			0.630	15.30	102.0	
			0.630	15.30	102.0	
			0.624	15.10	100.5	
Resteclin Aqueous Drops®	92.4 mg TC base/ml	13.86	0.570	13.80	99.6	100.0
			0.570	13.80	99.6	±0.6
			0.575	13.90	100.3	
			0.570	13.80	99.6	
			0.580	14.00	101.0	
Resteclin Syrup®	23.1 mg TC base/ml	13.86	0.570	13.75	98.8	99.4
			0.565	13.60	98.1	±1.2
			0.575	13.90	100.3	
			0.580	14.00	101.0	
			0.570	13.75	98.8	

(a) Tetracycline is abbreviated as TC.

Table XI cont'd...

Product	Labelled Drug Content	Theoretical conc. final dilution (µg/ml)	O.D.	Found conc. of final dilution (µg/ml)	% Labelled Strength	Mean
Declostatin Capsules®	250 mg DMCTC[a] HCl/capsule	14.72	0.390	14.40	97.8	
			0.395	14.50	98.5	97.7
			0.390	14.40	97.8	±0.7
			0.391	14.40	97.8	
			0.387	14.20	96.5	
Declomycin 300 Tablets®	300 mg DMCTC[a]/ tablet	14.70	0.388	14.40	97.8	
			0.380	13.90	94.4	96.3
			0.385	14.10	95.8	±1.4
			0.385	14.10	95.8	
			0.390	14.40	97.8	
Vibramycin Scripak Capsules®	100 mg doxycycline/capsule	17.31 µg hyclate/ cc	0.485	17.38	100.4	
			0.490	17.50	101.1	100.7
			0.485	17.38	100.4	±0.4
			0.485	17.38	100.4	
			0.490	17.50	101.1	
Vibramycin Oral Suspension®	5 mg doxycycline/cc (when constituted)	15.61 µg doxycycline monohydrate/ cc	0.410	15.88	105.8	
			0.405	15.63	104.2	105.7
			0.410	15.63	105.8	±0.9
			0.415	16.00	106.7	
			0.410	15.88	105.8	

(a) Demethylchlortetracycline abbreviated as DMCTC.

Table XI cont'd...

Product	Labelled Drug Content	Theoretical conc. final dilution (µg/ml)	O.D.	Found conc. of final dilution (µg/ml)	% Labelled Strength	Mean
Aureomycin Antibiotic Eye Ointment ®	10 mg CTC HCl/g	15.00	0.295	13.25	88.0	
			0.300	13.50	90.0	89.6
			0.310	14.00	93.3	±2.5
			0.290	13.00	86.7	
			0.300	13.50	90.0	
Aureomycin Surgical Powder ®	200 mg CTC[a] HCl/g	15.00	0.330	14.90	99.3	
			0.340	15.25	101.7	99.6
			0.330	14.90	99.3	±1.4
			0.335	15.00	100.0	
			0.325	14.65	97.7	
Aureomycin Ear Solution ®	50 mg CTC HCl/vial	15.00	0.335	15.00	100.0	
			0.330	14.90	99.3	99.7
			0.335	15.00	100.0	±0.3
			0.330	14.90	99.3	
Aureomycin Soluble Tablets ®	50 mg CTC HCl/tablet	15.00	0.340	15.25	101.7	
			0.335	15.00	100.0	100.2
			0.335	15.00	100.0	±1.6
			0.340	15.25	101.7	
			0.325	14.65	97.7	
Declomycin Syrup ®	73.6 mg DMCTC[b] / 5 cc	17.66	0.455	17.20	97.4	
			0.460	17.40	98.5	98.4
			0.462	17.50	99.1	±0.6
			0.459	17.40	98.5	
			0.460	17.40	98.5	

(a) Chlortetracycline abbreviated as CTC.

(b) Demethylchlortetracycline abbreviated as DMCTC.

Table XI cont'd...

Product	Labelled Drug Content	Theoretical conc. final dilution (µg/ml)	O.D.	Found conc. of final dilution (µg/ml)	% Labelled Strength	Mean
Achromycin Capsules ®	231.04 mg TC [a] base/capsule	13.86	0.575	1.390	100.3	99.6
			0.570	13.75	99.2	±0.7
			0.570	13.75	99.2	
			0.560	13.70	98.8	
			0.575	13.90	100.3	
Terra-Cortril Spray ®	60 mg OTC [b] /cc	15.00	0.440	13.14	87.6	
			0.450	13.42	89.5	88.2
			0.440	13.14	87.6	±1.0
Terra-Cortril Ear/ Eye Suspension ®	5 mg OTC/cc	25.00	0.520	24.85	99.4	
			0.520	24.85	99.4	98.9
			0.515	24.57	98.3	±0.6
			0.515	24.57	98.3	
			0.520	24.85	99.4	
Terra-Cortril Ointment ®	30 mg OTC/g	15.00	0.460	13.71	91.4	
			0.460	13.71	91.4	91.6
			0.465	13.85	92.5	±0.5
			0.460	13.71	91.4	
			0.460	13.71	91.4	
Aureomycin Capsules ®	250 mg CTC [c] HCl/capsule	15.00	0.340	15.25	101.7	
			0.330	14.80	98.7	
			0.345	15.50	103.3	101.1
			0.340	15.25	101.7	±1.8
			0.335	15.00	100.0	

(a) Tetracycline is abbreviated as TC.

(b) Oxytetracycline is abbreviated as OTC.

(c) Chlortetracycline is abbreviated CTC.

Table XII. Data Establishing Relationship Between

Tetracycline Phosphate Complex and Tetracycline Base

Aliquot of Stock Soln of TPC [a]	Conc. of TPC (µg/ml)	O.D. at 395 mµ	Reading for same conc. TC Base at 395 mµ	Ratio of O.D. of TPC/TC	Equiv. conc of TC [b] Base (µg/ml)
0.2 ml	5.0	.140	0.210	.666	3.39
0.4 ml	10.0	.280	0.415	.675	6.78
0.6 ml	15.0	.420	0.620	.677	10.17
0.8 ml	20.0	.570	0.825	.691	13.56
1.0 ml	25.0	.700	1.025	.683	17.15
				Av.=.678	

1.000 g of TPC =0.678 g TC base

(a) Tetracycline phosphate complex abbreviated as TPC.

(b) Tetracycline abbreviated as TC.

Table XIII. Data for Analysis of Dosage Forms Containing Tetracycline Phosphate Complex

Product	Labelled Drug Content	Theoretical Conc. of TPC[a] in Final Dilution (µg/ml)	O.D.	Found Conc. of TPC (µg/ml)	Equivalent Conc. of TC[b] Base (µg/ml)	% Labelled Strength	Average (%)
Tetrex Capsules®	TPC =250 mg TC HCl/cap	13.86	0.620	22.00	14.91	107.6	
			0.620	22.00	14.91	107.6	107.1
			0.615	21.80	14.78	106.6	±0.5
			0.620	2..00	14.91	107.6	
			0.615	21.80	14.78	106.6	
Tetrex-bid Capsules®	TPC =500 mg TC HCl/cap	13.86	0.615	21.80	14.78	106.6	
			0.610	21.50	14.57	105.1	106.7
			0.620	22.00	14.91	107.6	±1.0
			0.615	21.80	14.78	106.6	
			0.620	22.00	14.91	107.5	
Tetrex-APC Capsules®	TPC =125 mg TC HCl/cap	13.86	0.640	22.50	15.25	110.0	
			0.650	23.00	15.59	112.5	110.8
			0.640	22.50	15.25	110.0	±1.1
			0.640	22.50	15.25	110.0	
			0.645	22.80	15.45	111.5	
Azotrex Capsules®	TPC =125 mg TC HCl/cap	13.86	0.630	22.40	15.19	109.5	
			0.620	22.00	14.90	107.5	107.7
			0.615	21.80	14.78	106.6	±1.1
			0.620	22.00	14.90	107.5	
			0.620	22.00	14.90	107.5	

(a) Tetracycline phosphate complex abbreviated as TPC.
(b) Tetracycline abbreviated as TC.

Table XIII cont'd...

Product	Labelled Drug Content	Theoretical Conc. of TPC[a] in Final Dilution (µg/ml)	O.D.	Found Conc. of TPC (µg/ml)	Equivalent Conc. of TC[b] Base (µg/ml)	% Labelled Strength	Average (%)
Azotrex Syrup®	TPC =125 mg TC HCl/5 cc	13.86	0.560	20.00	13.56	97.8	
			0.560	20.00	13.56	97.8	96.8
			0.550	19.50	13.22	95.4	±1.3
			0.560	20.00	13.56	97.8	
			0.550	19.55	13.22	95.4	
Tetrex Syrup®	TPC =125 mg TC HCl/5 cc	13.86	0.610	21.50	14.50	104.6	
			0.610	12.50	14.50	104.6	103.9
			0.605	21.25	14.30	103.2	±1.0
			0.610	21.50	14.50	104.6	
			0.600	21.00	14.20	102.4	
Tetrex-AP Syrup®	TPC =125 mg TC HCl/5 cc	13.86	0.575	20.25	13.73	99.1	
			0.575	20.25	13.73	99.1	99.3
			0.590	20.80	14.10	101.7	±1.4
			0.575	20.25	13.73	99.1	
			0.570	20.00	13.56	97.8	

(a) Tetracycline phosphate complex abbreviated as TPC

(b) Tetracycline abbreviated as TC.